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ILLINOIS BIOLOGICAL MONOGRAPHS

Vol. IV October, 1917 No. 2

EDITORIAL COMMITTEE

STEPHEN ALFRED FORBES WILLIAM TRELEASE

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PUBLISHED UNDER THE
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THE GOLDFISH
(*Carassius carassius*)
AS A TEST ANIMAL IN THE STUDY OF TOXICITY

WITH GRAPHS AND TABLES

BY

EDWIN B. POWERS

Contributions from the
Zoological Laboratory of the University of Illinois
under the direction of Henry B. Ward, No. 109

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INTRODUCTION

The need of uniformity of remedial agents has long been recognized among therapeutists and pharmacologists. When the physiological activity of an agent is associated with or dependent upon its chemical constitution which can be determined by analysis the problem becomes a simple one. On the other hand when either the chemical constitution can not be determined or the activity of the substance is dependent upon some other factor independent of its constitution the problem becomes more complex. This difficulty has been overcome with more or less success by certain physiological assay methods, the history of which it is not deemed necessary to present as Baker (1913) has given an excellent review of the literature up to that date. Of the methods employed at present the most favored is Fagge and Stevenson's (1866) frog method as proposed by Houghton (1898) or some modification of it. Others have been suggested:—the blood pressure method, the guinea-pig method introduced by Laborde (1884) and further worked out by Reed and Vanderkleed (1908), the cat method of Hatcher and Brody (1910), the cockscomb method of Haskell (1914), the goldfish method of Pittenger and Vanderkleed (1915), and other methods.

The purpose of this investigation was to test the validity of the Pittenger and Vanderkleed goldfish method or to work out a usable modification of it. The problem then involved the development of a method and technic by which drugs or remedial agents, not readily assayed chemically, could be assayed or standardized physiologically by means of goldfish. In order to use the goldfish as test animals they must meet the following requirements: 1. They must be relatively constant in their reactions or resistance to the drug or agent to be standardized or at least there must be a constant seasonal rhythm. 2. They should be capable of being standardized with some standard of a conveniently assayable substance if seasonal rhythm is present. 3. They must be relatively sensitive to small variations in the concentration of the substance to be tested.

MATERIALS AND METHODS OF STUDY

A series of experiments was run with the goldfish to determine how far they would comply with the three requirements just mentioned. In order to do this the goldfish were tested with a number of substances varying in toxic activities. A number of solutions of each substance tested were prepared of which the concentration of each differed only 10% or less from that of the one next to it in the series. Two goldfish of known weights were then placed in two liters of each of the solutions contained in a three liter wide-mouth bottle. The survival time of each fish in each solution was noted and recorded. The bottles were kept stoppered when volatile substances

were being tested. The stock of fish was kept at as nearly constant temperature as possible by the addition of hot water to the aerated tap-water. The experiments were run at the same temperature as that of the stock by placing the experimental bottles in the stock tank. This eliminated any ill effect due to the sudden change of temperature (Wells 1914). By these experiments it was hoped to determine two things: (1) the fitness of the goldfish as a test animal and (2) the most adaptable range of survival time of the goldfish for pharmacodynamic assay work.

The goldfish used in these experiments were *Carassius carassius* L. (Meek and Hilderbrand 1910), the Crucian carp or goldfish. This is the goldfish commonly sold for aquaria. *C. auratus* is less common and was not obtainable for this work.

ACKNOWLEDGMENTS

The author desires to acknowledge his indebtedness to Dr. J. H. Beal, Director of Pharmaceutical Research, who suggested the following research and supplied the necessary funds for its conduct, as well as many helpful suggestions throughout the course of the work.

The author also extends his thanks to Dr. Victor E. Shelford, in whose laboratory the experiments were made, for much valuable advice and assistance and to Dr. C. H. Sisam for suggestions on mathematical points, for the application of which, however, the author assumes sole responsibility. The author's thanks are also due to Drs. Geo. D. Beal, H. B. Lewis, T. R. Ball, and H. E. Eastlack for frequent help in chemical problems.

EXPERIMENTAL DATA

In the study of the goldfish as a test animal it was deemed advisable to determine the uniformity or lack of uniformity of the resistance or susceptibility of the goldfish first to certain of the less toxic simple substances; then later to take up a study of the more toxic and the more complex substances. First the chlorides and nitrates of the alkali and alkaline earth metals were employed. Later the heavier metals, cupric chloride, cadmium chloride, and ferric chloride, and finally hydrochloric acid, potassium cyanide, methyl, ethyl, and isobutyl alcohols, phenol, caffeine, and pyridine were used. It was found that all substances tested, with the exception of $CuCl_2$, $CdCl_2$, and to a certain extent $FeCl_3$, have certain points in common when the toxic activity of varying amounts of the substance tested is considered. This can be best illustrated by a detailed study of one or two representative substances and by comparing these with the other substances employed. The experiments with lithium chloride will first be considered.

In the lithium chloride experiments the resistance of the goldfish to concentrations varying from 0.089 N. to 0.466 N. was tested. By referring to Table I it will be found that the goldfish died fairly uniformly in any given concentration of the lithium chloride solution. For other chlorides and nitrates see Tables II to XV. The 2.5 g. goldfish (Table III) having a survival time of 135 minutes and marked with an asterisk (*) was taken out of the sodium chloride solution for dead, but when placed in an HCl solution

TABLE I

LITHIUM CHLORIDE. TEMPERATURE 21° C. DECEMBER 1, 1916

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
0.466	2.8	36	0.200†	2.8	141	Daggers (†) indicate the range in which the greatest difference in survival times occured in any two solutions with the least difference in concentrations in which the fish died uniformly.
"	3.2	41	"	3.05	179	
0.388	3.0	46	0.166	2.8	234	
"	4.2	44	"	3.6	310	
0.322†	2.9	54	0.133	2.9	525	
"	3.2	58	"	3.4	925	
0.266†	2.9	92	0.111	3.0	1204	
"	3.2	96	"	3.2	1214	
0.222†	3.3	116	0.089	2.7	±1320	
"	3.35	112	"	3.0	1620	

showed very slight signs of life. All goldfish when taken out of a solution for dead were tested by placing them in a hydrochloric acid solution and if any signs of life were evidenced the goldfish was designated by an asterisk in experimental data as not dead. No corrections have been made either in this or subsequent tables or graphs since it was deemed as being within experimental error as well as within individual variation of the goldfish. For further discussion of the uniformity of the survival time of the goldfish see page 40. By a close examination of data of killing goldfish in different concentrations of lithium chloride (Table I) it is found that the survival time of

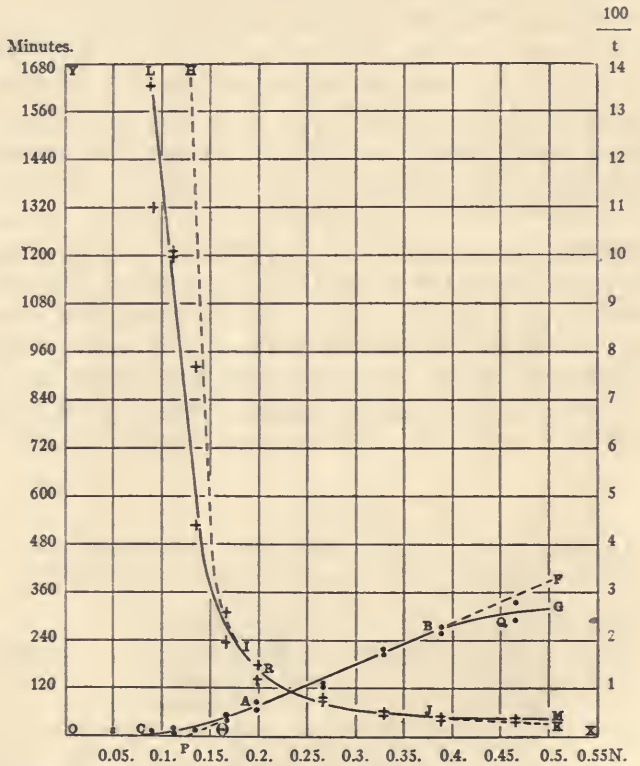

Figure 1. Lithium chloride. LIJM is the survival time curve. The survival time of the goldfish is plotted as ordinate and the concentration of the LiCl as abscissa. The crosses (+) are located from actual experimental data. CABG is the velocity of fatality curve. The concentration of the LiCl is plotted as abscissa and the reciprocal of the survival time in minutes or the velocity of fatality is plotted as ordinate. Instead of 1 over the survival time which gives the reciprocals, 100 over the survival time is used to avoid the use of fractions. The circles (●) are located by calculations from actual experimental data. The numbers at the left hand of the graphs represent minutes of survival time and those at right hand side of graphs represent units of velocity of fatality. The line PABF is the theoretical velocity of fatality curve and is a straight line. HIJK is the theoretical survival time curve.

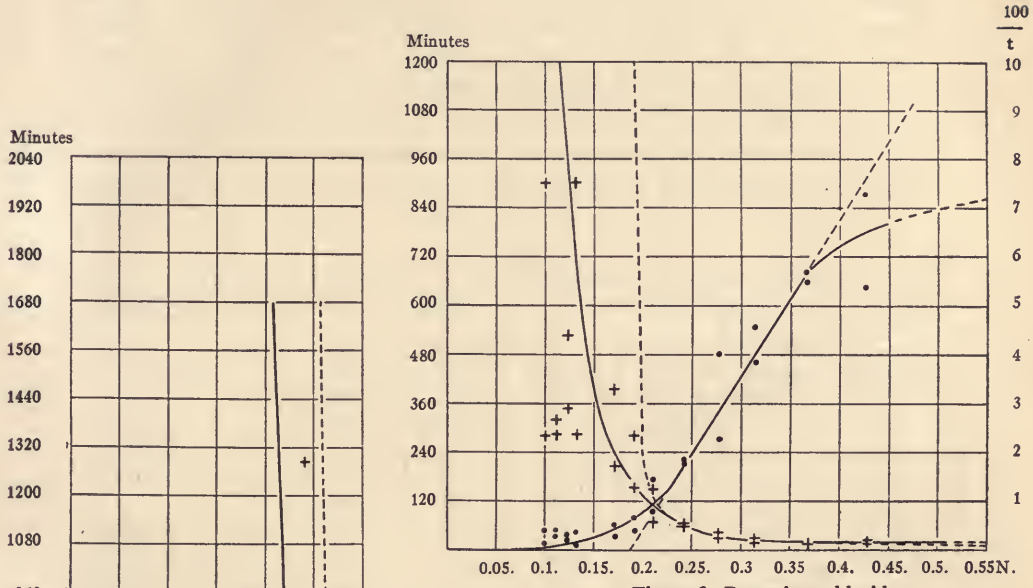

Figure 3. Potassium chloride

Figure 2. Sodium chloride

the goldfish does not increase in the same ratio as the concentration of the lithium chloride decreases, i. e., the survival time of the the goldfish is not in inverse proportion to the concentration of the lithium chloride employed. At higher concentrations (0.466, 0.388, and 0.322 N.) the increase in survival time of the goldfish was less rapid than the decrease in concentration of the solution. With concentrations from 0.322 to 0.133 N. there was a more rapid increase in survival time of the goldfish in proportion to the decrease in concentration of the solutions. And finally with concen-

trations from 0.133 to 0.089 N. there was again a less rapid increase in survival time in proportion to the decrease in concentration of the lithium chloride solutions. These points can be better illustrated by the graphic method, (Fig. 1). Let one block abscissa represent 0.05 N. LiCl and one block ordinate represent 120 minute survival time of the goldfish and plot data as represented in Table I. When the points (+) have been located on the graphs the curve LIJM can be drawn by interpolation. The crosses of this and all subsequent graphs are located from actual experimental data. The curve LIJM thus

TABLE II

SODIUM CHLORIDE. TEMPERATURE 21.5° C. NOVEMBER 19 TO 20, 1916

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
0.600	2.5	28	0 277†	2.6	±178	
"	2.7	30	"	2.7	260	Daggers (†) as in Table I. Fish were all alive in 0.18, 0.15 and 0.124 N. solutions after 10200 minutes when experiments were discontinued.
0.500†	2.7	33	0.266	2.6	±270	
"	2.7	34	"	2.7	296	
0.417†	2.85	61	0.253	2.7	457	
"	2.9	55	"	2.8	304	
0.347†	2.6	97	0.241	2.65	478	
"	2.7	93	"	2.7	1290	
0.289†	2.75	114	0.201	?	2040	
"	2.8	155	"	3.0	9240	

TABLE III

SODIUM CHLORIDE. TEMPERATURE 21.5° C. APRIL 10, 1917

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
0.600	2.7	35	0.278†	3.3	134	
"	3.8	38	"	3.4	129	
0.500†	2.5	35	0.263	2.4	130	
"	2.9	37	"	2.5	141	Daggers (†) as in Table I. *Fish was not dead when taken out of solution.
0.417†	2.5	40	0.253	2.4	255	
"	3.2	42	"	2.5	135*	
0.347†	2.3	56	0.241	2.5	295	
"	2.5	68	"	2.9	400	
0.289†	2.7	115	0.200	2.4	±1020	
"	2.9	125	"	3.0	±1020	

TABLE IV

POTASSIUM CHLORIDE. TEMPERATURE 20.5° C. NOVEMBER 17, 1916

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
0.434	2.9	14	0.192	2.85	151	
"	3.2	17	"	2.9	282	
0.378	2.9	11	0.172	3.0	207	
"	3.0	16	"	3.1	396	
0.328†	2.8	22	0.134	2.9	286	Daggers (†) as in Table I. *Fish was not dead when taken out of solution.
"	3.0	26	"	2.9	±900	
0.286†	3.2	25*	0.122	2.8	347	
"	3.3	44	"	3.1	527	
0.243†	2.9	55*	0.112	3.1	318	
"	2.8	57	"	3.2	292	
0.214†	2.9	69	0.102	3.55	279	
"	2.8	150	"	2.8	±900	

drawn resembles that of an hyperbola and has been designated as the survival time curve the nature of which can be better illustrated by drawing the reciprocal curve. In the reciprocal curve the reciprocal of the survival time of the goldfish is plotted as ordinate. The normality is plotted as in the survival time curve. To avoid the use of fractions 100 over the survival time is taken as the reciprocal. One block ordinate represents one unit reciprocal. The circles (●) represent the location of the reciprocals. The curve CABG is drawn by interpolation and has been designated as the velocity of fatality curve. By an examination of this curve it is seen that it rises very slowly from the point C to A, i.e., in low concentrations, more rapidly from A to B, i.e., in higher concentrations, and again there is a falling off from B to G at still higher concentrations. By a closer examination of the curve it is seen that the portion from A to B approaches a straight line and for all practical purposes this portion can be considered as such. The point C where this curve cuts the X-axis has been designated as the actual threshold of toxicity concentration, i.e., the concentration below which the substance will not kill the goldfish. The portion of the curve from A to B is not a straight line but approaches a straight line in that the direction of curvature is being reversed from that of the curvature from C to A to that of the curvature from B to G (See velocity of fatality curves, Figures 1 to 21.) For a further discussion of the velocity of fatality curve see pages 48, 52. The portion from A to B of the curve CABG if considered as a straight line and prolonged cuts the X-axis at the point P. The curve PABF thus drawn has been designated as the theoretical velocity of fatality curve and the point P has been designated as the theoretical threshold of toxicity

concentration. The curve HIJK is an equilateral hyperbola drawn from calculations and has been designated as the theoretical survival time curve. The two curves HIJK and LIJM show the variation of the actual survival

TABLE V

AMMONIUM CHLORIDE. TEMPERATURE 21° C. NOVEMBER 24 TO 25, 1916

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
0.352	3.5	29	0.141	3.25	357	
"	3.7	20	"	3.4	±600	
0.319	3.6	30	0.115	3.15	320	
"	3.8	31	"	3.9	297	
0.268†	3.45	38	0.096	3.2	547	
"	4.0	37	"	3.2	±1020	Daggers (†) as in Table
0.249†	2.8	48	0.064	3.1	±1020	I.
"	3.2	64	"	3.3	±1020	
0.224†	2.5	99	0.032	3.1	376	
"	3.7	90	"	3.4	1080	
0.166	2.9	283				
"	3.2	285				

TABLE VI

AMMONIUM CHLORIDE. TEMPERATURE 21.5° C. APRIL 12, 1917

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
0.351	2.1	17	0.160	2.8	166	
"	4.3	28*	"	3.7	261	
0.320	2.8	26	0.141	2.3	111	Daggers (†) as in Table
"	3.0	22	"	?		I. *Fish was not dead
0.268†	3.0	48	0.115	3.0	376	when taken out of solu-
"	3.2	40	"	3.5	106	tion.
0.257†	2.7	51	0.096	2.7	123	** Fish was alive after
"	2.9	43	"	2.7	663	5040 minutes when
0.224†	2.8	53	0.064	2.5	±1080	experiment was discon-
"	2.8	65	"	2.8	±1080	tinued.
0.186	2.5	141	0.041	2.5	±1080*	
"	2.8	95	"	?	———**	

time curve from that of an equilateral hyperbola. To summarize: in the lithium chloride and all toxic substances tested with the exception of $CuCl_2$, $CdCl_2$, and to a certain extent $FeCl_3$ the following conditions were met:— 1. A concentration (the point C, about 0.073 N. LiCl), the threshold of toxicity concentration, was found below which the goldfish were not killed. 2. Just above the threshold of toxicity concentration the rate of fatality, as expressed by the reciprocal of the survival time, increased very slowly with increase in concentration of the toxic substance employed. This is represented by the portion C to A, about 0.073 to 0.19 N. LiCl, of the velocity of fatality curve CABG (Fig. 1). 3. With higher concentrations, concentrations

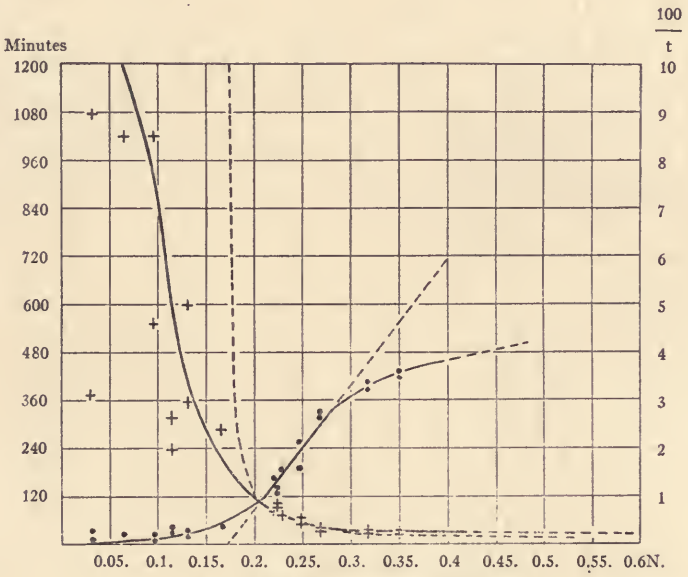

Figure 4. Ammonium chloride

sufficient to kill the goldfish in not less than 54 to 58 minutes and not more than 234 to 310 minutes in lithium chloride solutions, the velocity of fatality increased more rapidly in proportion to the increase in concentration of the toxic substance, and this portion of the velocity of fatality curve approaches a straight line. (The portion A to B, curve CABG, Fig. 1.) Table XXIV shows minimum and maximum survival time of goldfish and concentration of toxic substances tested where its velocity of fatality curve approaches a straight line. The daggers (†) in Tables I to XXIII also indicate the concentrations of the substances and the survival time of the goldfish where their velocity of fatality curves approach a straight line. 4. At still higher concentrations (0.375 to 0.45 N. LiCl) the increase in the velocity of fatality was again less rapid in proportion to the increase in concentration of the

TABLE VII

SODIUM NITRATE. TEMPERATURE 21° C. DECEMBER 9, 1916

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
0.500	2.7	61	0.260†	3.0	134	Daggers (†) as in Table I.
"	2.9	164	"	3.4	274	
0.460	2.7	116	0.220†	2.6	171*	*Fish was not dead when taken out of solution.
"	2.7	118	"	2.7	196	
0.440	2.65	148	0.180	2.6	433	
"	3.2	68	"	3.0	656	Fish in 0.12 N. and 0.10 N. solutions were alive after 1680 minutes when experiments were discontinued.
0.380†	2.9	122*	0.150	2.5	593*	
"	3.1	109	"	2.9	±840	
0.310†	3.05	133				
"	3.4	180				

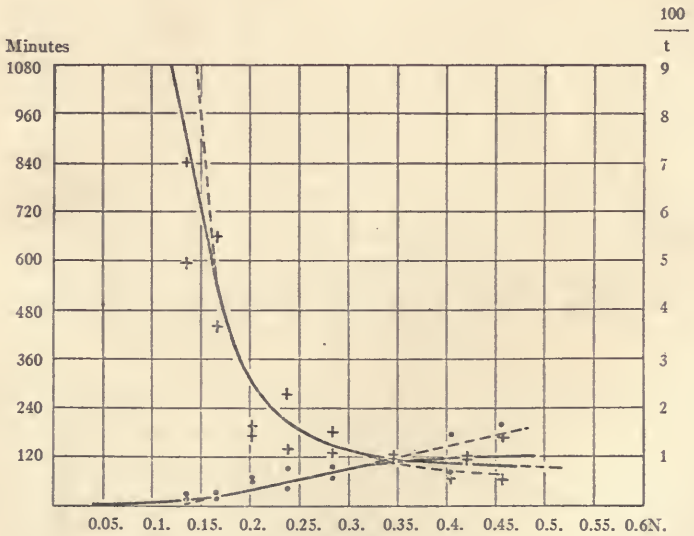

Figure 5. Sodium nitrate

solution. See the portion B to Q (Fig. 1). 5. At the highest concentrations tested the velocity of fatality curve a second time approaches a straight line but with a less rapid increase of velocity of fatality in proportion to the concentration of the solution than the portion of the velocity of fatality curve represented by the portion A to B, curve CABG (Fig. 1). (The portion Q to G,

Fig. 1.) This last point is better illustrated by the curves represented in Figures 2 to 20. These curves are logarithmic in nature. See pages 48, 51 for discussion of the theoretical equation for the velocity of fatality curve.

TABLE VIII

AMMONIUM NITRATE. TEMPERATURE 21° C. DECEMBER 18, 1916

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
						Daggers (†) as in Table I.
0.309	1.95	29*	0.120	2.0	±720	*Fish was not dead when taken out of solution.
"	2.4	32	"	2.3	±2160	
0.253†	2.3	37	0.100	2.3	±1080	
"	2.7	52	"	2.4	±1800	*[1]A close approximate survival time.
0.213†	2.4	78	0.080	1.7	1620	
"	2.4	82*	"	1.9	1800	*[2] Fish was alive after 4 days when taken out of solution and experiment discontinued.
0.173	2.1	228*[1]	0.066	1.7	5625	
"	2.5	228*[1]	"	1.7	——*[2]	
0.140	2.3	371	0.053	2.2	5400*[1]	
"	?	404	"	2.0	——*[2]	Fish were alive after 4 days in 0.033 N. Solution when experiment was discontinued.

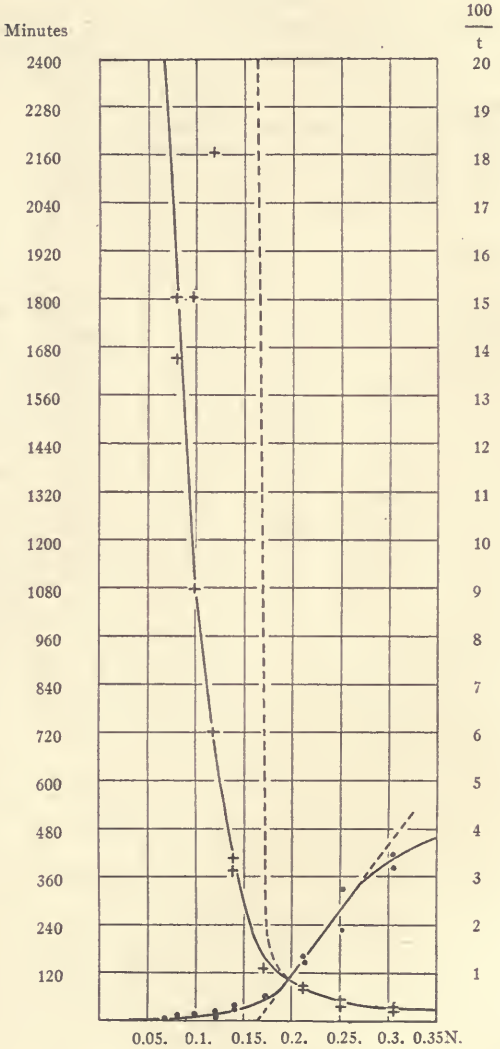

Figure 6. Ammonium nitrate

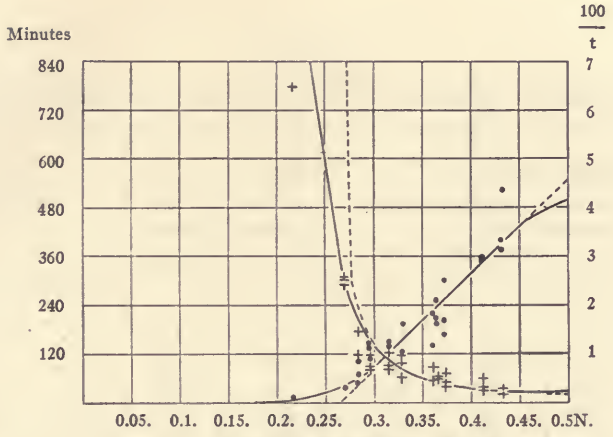

Figure 7. Magnesium chloride

TABLE IX

MAGNESIUM CHLORIDE. TEMPERATURE 20.5°C. NOVEMBER 8 TO 14, 1916

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
2.158	4.4	10.5				
0.432†	3.3	23	0.298	3.2	82	
"	4.1	34	"	3.4	91	
"	5.0	30*	"	3.6	86	
0.411†	4.2	33	0.283	3.6	117	
"	5.4	35	"	5.1	174	
"	6.2	58	"	6.3	270	Daggers (†) as in Table I.
0.373†	3.4	40	0.270	3.7	183	
"	3.7	45	"	5.5	132	*Fish was not dead when taken out of solution.
"	5.8	70	"	6.0	239	
0.360†	3.9	42	0.257	3.3	306	
"	5.7	57	"	3.7	291	
"	6.1	61	"	4.3	301	
0.328†	3.4	62	0.216	5.4	780	
"	3.9	118				
"	7.6	99	"	4.3	1200	
0.313†	4.2	121	0.142	?	30420	
"	5.0	88	"	?	15180	
"	5.5	80	"	?	4680	

TABLE X

CALCIUM CHLORIDE. TEMPERATURE 21°C. NOVEMBER 21 AND DECEMBER 2, 1916

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
0.583	2.6	43	0.213	2.3	136	
"	2.6	43	"	2.5	±900	
0.356†	1.9	48*	0.202	?	±1080	
"	2.05	59	"	3.1	±1920	Daggers (†) as in Table I.
0.347†	2.6	57*	0.171	2.3	2640	
"	2.7	66*	"	2.5	4080	*Fish was not dead when taken out of solution.
0.299	2.0	65	0.168	2.8	6627	
"	2.2	90	"	3.1	±2760	
0.290†	2.8	91	0.142	2.5	1391	*[1]Experiment discontinued after 4320 minutes. Fish were all alive.
"	3.0	139	"	3.0	4331	
0.250†	2.7	182	0.140	2.9	1920	
"	2.9	111	"	3.4	4320	
0.249†	2.3	174	0.128	—	*[1]	
"	2.25	190	"	—	*[1]	

TABLE XI

STRONTIUM CHLORIDE. TEMPERATURE 20°C. NOVEMBER 25, 1916

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
0.458	3.4	50	0.289†	3.3	80	
"	2.6	59	"	4.1	88	
0.380	2.8	46	0.237†	3.6	168	Daggers (†) as in Table I.
"	3.75	51	"	3.6	347	
0.318†	3.4	59	0.193	2.7	±1020	
"	3.5	76	"	3.4	±1860	

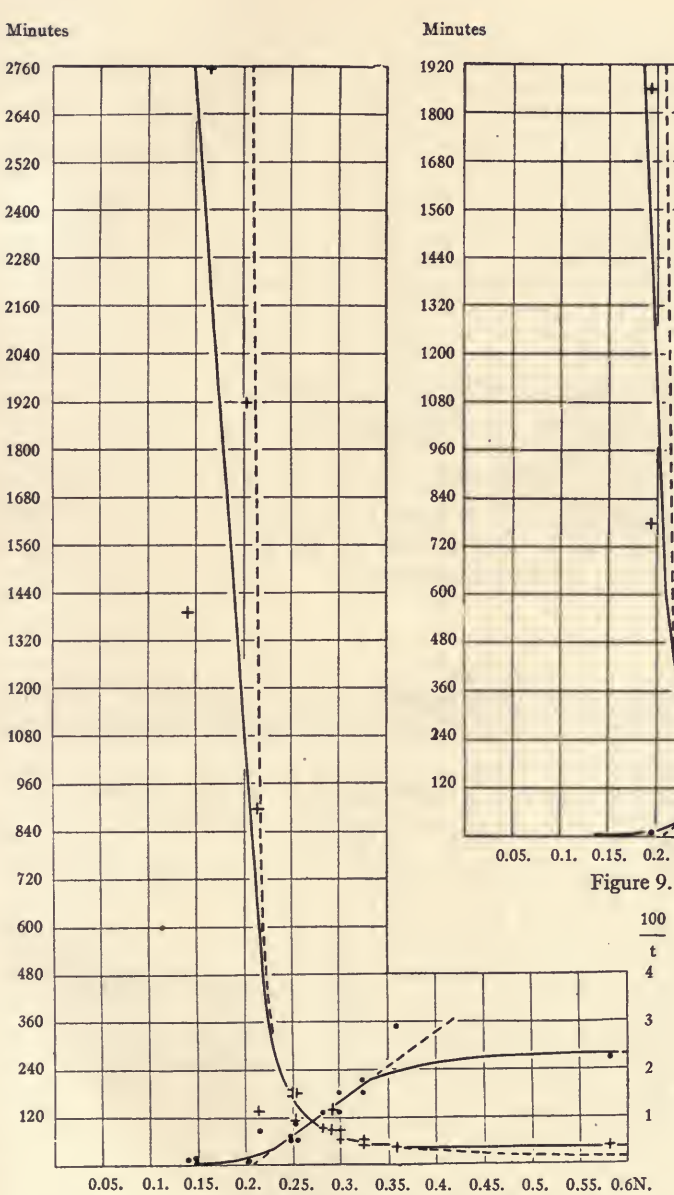

Figure 8. Calcium chloride

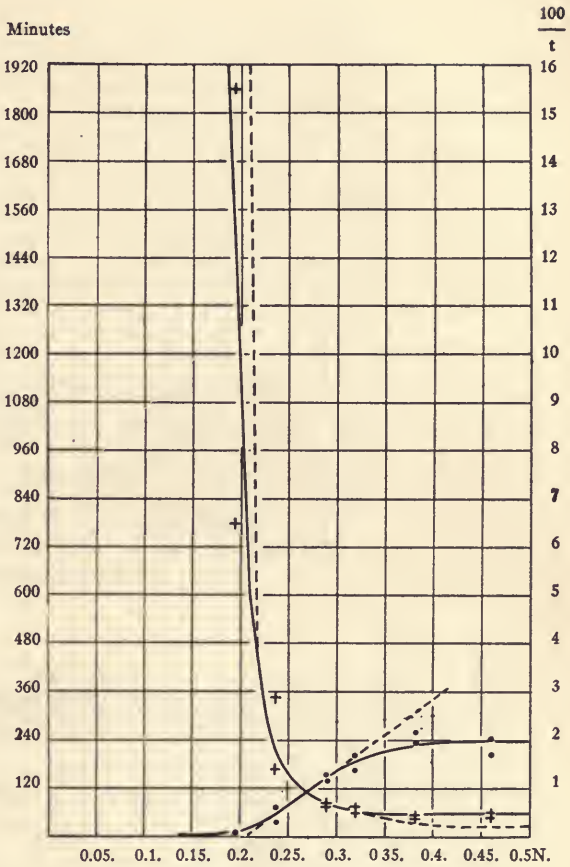

Figure 9. Strontium chloride

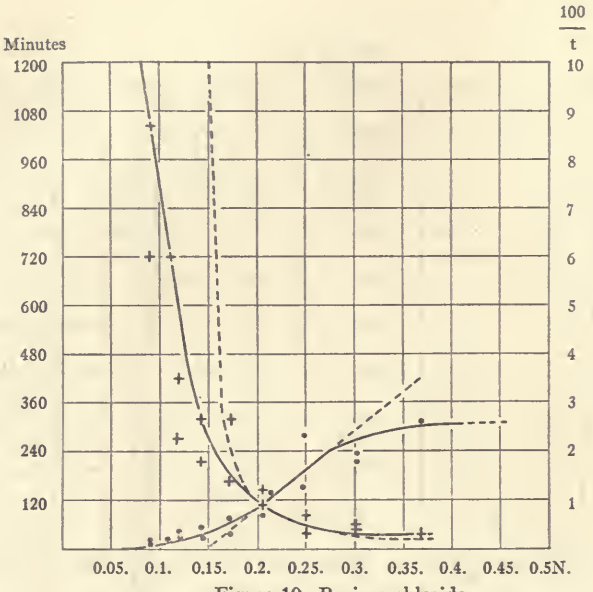

Figure 10. Barium chloride

TABLE XII

BARIUM CHLORIDE. TEMPERATURE 20.5°C. NOVEMBER 22, 1916

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
0.368	3.0	38	0.143	3.35	216	
"	3.2	38	"	3.7	314	
0.302	3.2	51*	0.119	3.3	±420	Daggers (†) as in Table I.
"	3.2	56	"	3.7	±272	
0.249†	3.5	43	0.107	3.5	±720	*Fish was not dead when taken out of solution.
"	3.5	80	"	3.6	±720	
0.208†	3.35	113	0.090	3.2	±720	
"	3.5	144	"	3.8	1020	
0.172†	2.8	169				
"	3.7	316				

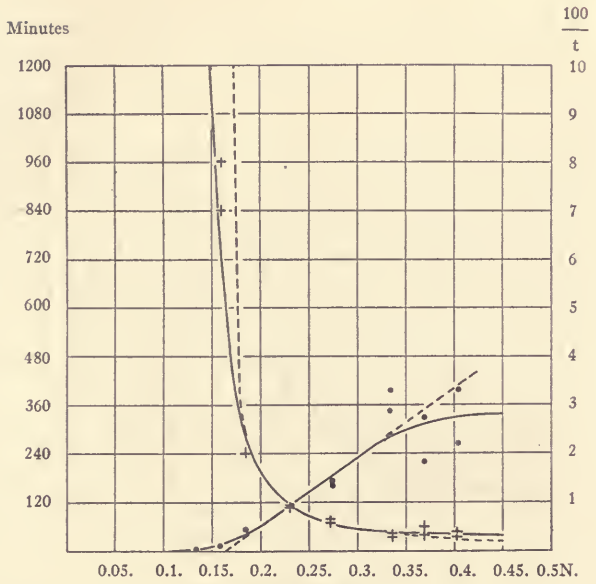

Figure 12. Magnesium nitrate

TABLE XIII

MAGNESIUM NITRATE. TEMPERATURE 21°C. DECEMBER 15 AND 16, 1916

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
						Daggers (†) as in Table I.
0.405	2.7	33*	0.229†	2.2	107*	*Fish was not dead when taken out of solution. *[1]Fish had been dead possibly a few minutes. Fish were alive in 0.132 N. 0.106 N. 0.088 N. and 0.07 N. solutions after 3120 minutes when experiments were terminated.
"	2.7	46*[1]	"	2.9	104*	
0.370†	2.6	37*	0.185	2.4	±240	
"	2.7	56*	"	2.6	±240	
0.334†	2.4	33*	0.158	1.9	±840	
"	2.5	35*	"	2.5	±960	
0.273†	2.9	70*				
"	3.4	73*				

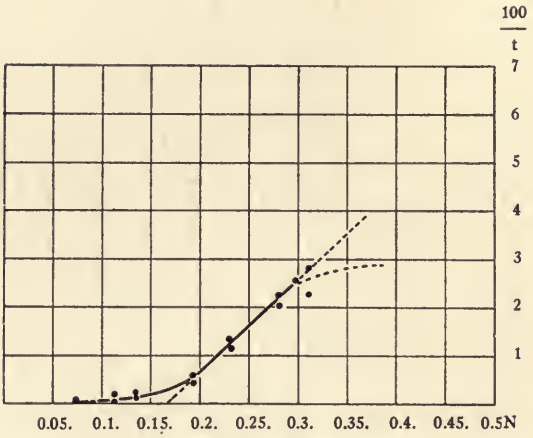

Figure 13. Calcium nitrate

TABLE XIV

CALCIUM NITRATE. TEMPERATURE 21°C. DECEMBER 16, 1916

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
0.311	2.6	42	0.133	2.0	±840	
"	3.0	44	"	2.1	±840	
0.281†	2.2	45	0.111	2.4	±1200	
"	2.4	50	"	2.4	±1200	Daggers (†) as in Table I.
0.229†	2.4	75	0.088	2.4	2116	
"	2.4	85	"	2.2	4936	
0.192†	2.6	186	0.074	2.5	2580	
"	2.6	186	"	2.0	2920	

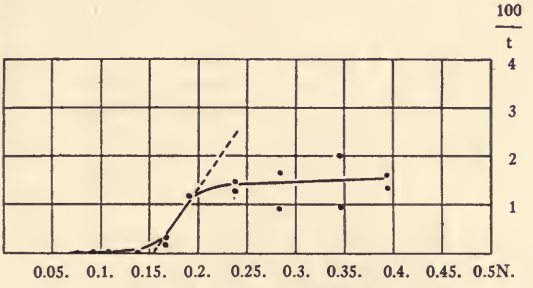

Figure 14. Strontium nitrate

TABLE XV

STRONTIUM NITRATE. TEMPERATURE 21°C. DECEMBER 14, 1916

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
0.394	2.7	63	0.165†	3.5	±300	
"	2.5	75	"	3.5	600	
0.347	2.7	108	0.137	2.9	435	Daggers (†) as in
"	2.9	50	"	3.4	±990	Table I.
0.283	3.0	60	0.110	2.2	816	Fish were alive in
"	3.2	111	"	3.5	6240	0.073 N. solution after
0.238	3.0	70	0.091	3.2	1923	5760 minutes when ex-
"	2.8	85	"	2.8	8760	periment was termin-
0.192†	3.0	92				ated.
"	3.2	87				

TABLE XVI

POTASSIUM CYANIDE. TEMPERATURE 21.5° C.

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes
0.2×10^{-1}	2.9	35*	0.5×10^{-4}	2.7	118
"	4.5	59	"	3.0	94
0.12×10^{-1}	3.1	61	0.375×10^{-4}	3.0	138
"	3.4	96	$0.25\dagger\times10^{-4}$	3.1	146*
0.6×10^{-2}	3.7	96	"	3.5	204
"	4.3	64	$0.235\dagger$		
			$\times10^{-4}$	2.9	192
0.375×10^{-2}	3.3	67	"	3.6	163
"	3.3	68	$0.22\dagger\times10^{-4}$	2.3	128
0.2×10^{-2}	2.8	69	"	4.2	179
"	4.1	97	$0.217\dagger$		
			$\times10^{-4}$	2.5	138*
0.1×10^{-2}	3.6	43*	"	3.7	253
"	4.3	71	$0.215\dagger$		
			$\times10^{-4}$	3.8	296
0.5×10^{-3}	3.4	123	"	3.8	1658
"	3.7	90	$0.211\dagger$		
			$\times10^{-4}$	2.8	178
0.25×10^{-3}	3.4	98	"	3.9	928
"	4.2	98	$0.205\dagger$		
			$\times10^{-4}$	2.9	2135
0.162×10^{-3}	2.7	84	"	3.8	1945
"	3.0	84	0.195×10^{-4}	2.8	505
0.135×10^{-3}	3.2	84	"	3.2	2785
"	3.5	84	0.185×10^{-4}	3.1	715
0.1×10^{-3}	3.3	70*	"	4.2	655
"	3.6	67*	0.175×10^{-4}	2.4	1519
0.85×10^{-4}	2.9	79	"	4.2	$\pm$1020
"	2.95	79	0.12×10^{-4}	2.9	2615
0.7×10^{-4}	2.6	82	"	3.0	7080
"	2.9	74*			

Daggers (†) as in Table I

*Fish was not dead when taken out of solution

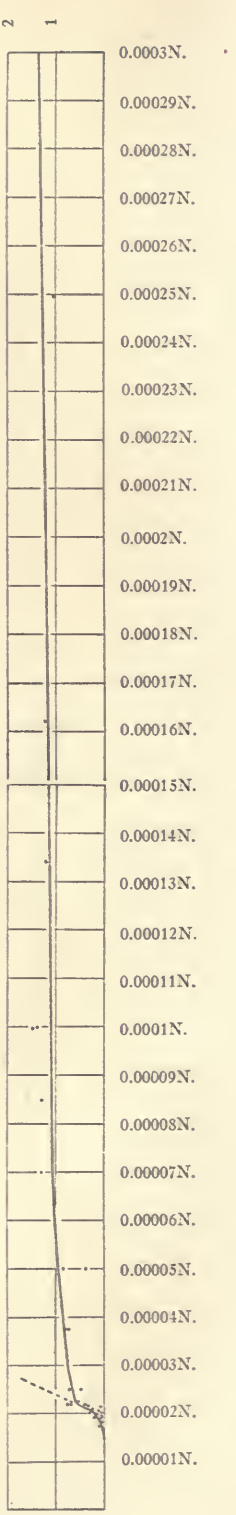

Fig. 14 (at right). Potassium cyanide. Velocity of fatality curve only

TABLE XVII

HYDROCHLORIC ACID. TEMPERATURE 21.5° C.

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes
8.89×10^{-3}	2.8	27*	$0.31\dagger\times10^{-3}$	3.2	353
"	3.4	34	"	4.0	246
7.32×10^{-3}	3.0	22*	0.209×10^{-3}	4.5	±1000
"	5.7	33*	"	5.5	±1000
4.19×10^{-3}	2.8	30	0.125×10^{-3}	3.4	±1200
"	5.0	36	"	4.0	±2520
2.09×10^{-3}	3.0	37	8.37×10^{-5}	3.5	±1200
"	5.1	40	"	5.0	±2520
1.05×10^{-3}	3.0	50*	6.27×10^{-5}	5.0	±1200
"	5.8	70	"	4.8	660
$0.9\dagger\times10^{-3}$	3.2	46*	4.18×10^{-5}	4.7	±1200
"	4.0	72*	"	4.1	697
$0.774\dagger\times10^{-3}$	2.2	78*	3.13×10^{-5}	5.1	±1200
"	3.3	87	"	5.2	±2420
$0.627\dagger\times10^{-3}$	4.7	98	2.09×10^{-5}	3.3	553*
"	4.3	110	"	4.1	±1200
$0.523\dagger\times10^{-3}$	3.9	134	1.57×10^{-5}	5.7	584
"	4.1	161	"	3.9	794
$0.418\dagger\times10^{-3}$	4.2	339			
"	5.2	480*			

Daggers (†) as in Table I

*Fish was not dead when taken out of solution

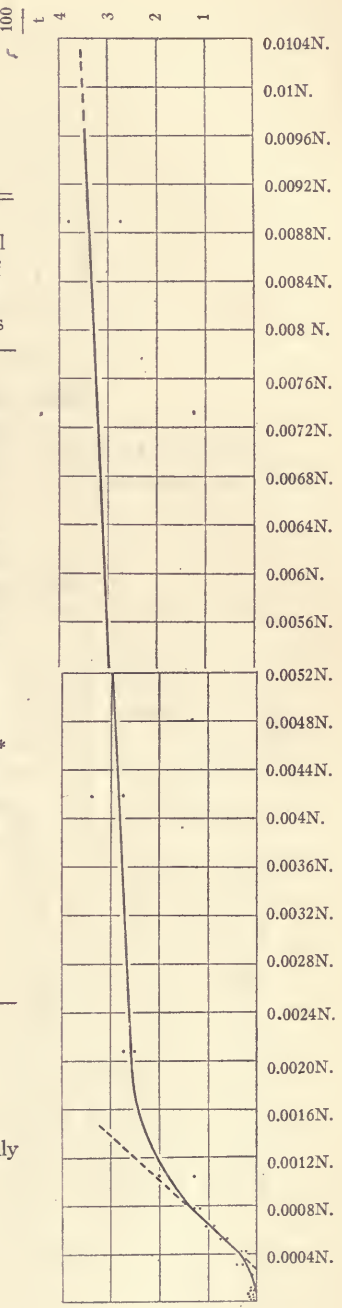

Fig. 15 (at right). Hydrochloric acid. Velocity of fatality curve only

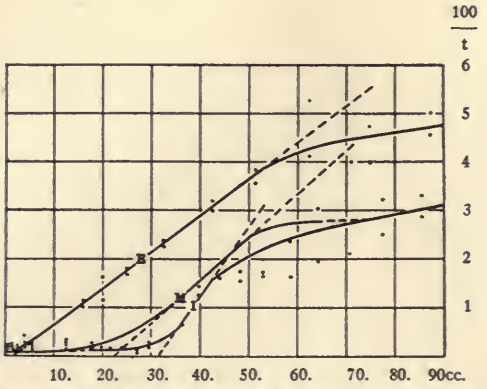

Figure 16. Methyl alcohol (M); Ethyl alcohol (E); Iso-butyl alcohol (I). Velocity of fatality curves only.

TABLE XVIII

METHYL ALCOHOL. TEMPERATURE 21.5° C.

cc. per l.	Weight of fish in grams	Survival time of fish in minutes	cc. per l.	Weight of fish in grams	Survival time of fish in minutes	Remarks
62.5	2.3	36*	6.0	2.8	603	
"	3.4	38*	"	4.0	258	
52.5	2.3	40*	4.0	3.6	517	
"	3.2	45	"	4.2	502	
42.5†	2.0	52	2.5	2.4	245	
"	3.6	57*	"	2.5	534	Daggers (†) as in Table I.
32.5†	3.6	±109	1.5	3.2	651	
"	4.2	±109	"	3.4	906	*Fish was not dead when taken out of solution.
25.0	3.0	206	1.0	2.9	507	
"	4.2	32	"	3.8	±906	
20.0	3.4	271	0.5	3.7	581	
"	3.4	421	"	5.0	901	
16.0	2.8	151	0.25	2.4	791	
"	3.8	299	"	3.2	646	
12.5	3.0	163				
"	2.4	325				

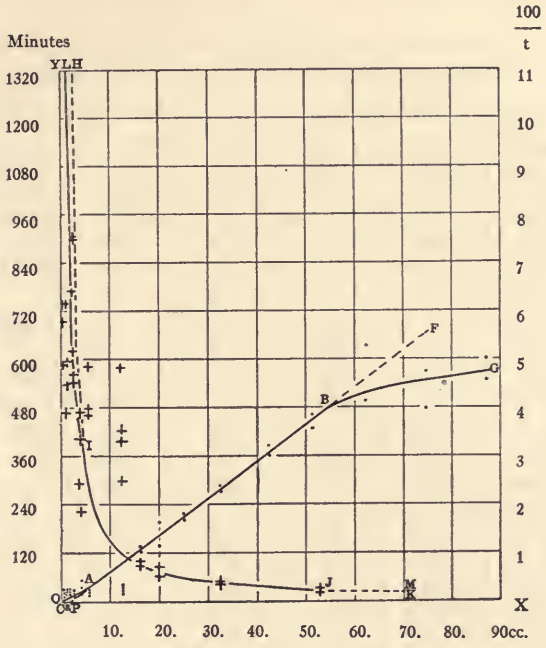

Figure 17. Ethyl alcohol

TABLE XIX

ETHYL ALCOHOL. TEMPERATURE 21.5° C.

cc. per l.	Weight of fish in grams	Survival time in minutes	cc. per l.	Weight of fish in grams	Survival time in minutes	Remarks
87.5	2.7	20	6.0	3.2	480	
"	3.3	22	"	3.3	475	
75.0	3.1	21*	"	2.6	462	
"	3.3	25	"	4.1	582	
62.5	3.0	19*	4.0	3.0	469	
"	3.3	24	"	4.5	403	
51.5†	2.8	26*	"	2.9	222	
"	3.5	28	"	3.0	290	Daggers (†) as in Table I.
42.5†	3.5	31	2.5	3.5	±900	
"	3.9	33*	"	4.0	±900	*Fish was not dead when taken out of solution.
32.5†	3.3	42	"	3.2	±540	
"	3.5	44*	"	5.0	620	
25.0†	2.8	56	"	3.4	561	
"	4.0	59*	"	5.0	771	
20.0†	2.5	61	1.5	2.8	593	
"	3.9	85	"	3.4	538	
16.0†	3.2	88*	1.0	2.4	738	
"	2.5	98*	"	2.5	465	
12.5	3.6	299	0.5	2.4	738	
"	4.3	579	"	3.3	583	
"	2.2	397	0.25	3.0	396	
"	3.4	472	"	3.0	694	

TABLE XX

Iso-Butyl Alcohol. Temperature 21.5°C.

cc. per l.	Weight of fish in grams	Survival time of fish in minutes	cc. per l.	Weight of fish in grams	Survival time of fish in minutes	Remarks
14.	3.2	20	6.0	2.6	43*	Boiling point 102° to 104° C.
"	3.4	25	"	2.8	14*	
12.5	2.9	24	5.0	3.3	64	
"	3.2	30	"	3.9	78*	Daggers (†) as in Table I.
11.0	2.9	28*	4.0	2.8	101*	
"	3.2	32	"	5.5	130*	*Fish was not dead when taken out of solution.
9.0	2.9	33	3.25	3.3	464	
"	3.5	36*	"	3.4	654	
7.5	2.9	41*	2.5	2.8	1680	
"	5.5	46	"	4.2	900	
20.0	3.0	17	4.4	3.0	57*	Boiling point 104° to 105° C.
"	4.0	18	"	4.9	63*	
16.0	2.4	23	4.0	3.8	70	
"	3.3	29	"	3.9	80*	
12.5	3.0	29	"	3.6	70*	
"	4.0	29	"	4.5	83	
9.0	3.4	35	3.62	2.3	157*	
"	4.2	39*	"	3.5	162	
8.55	4.2	30*	3.3	2.6	302	
"	4.5	35*	"	3.9	328*	
7.77	4.3	31	2.97	3.2	628	
"	4.4	40	"	5.0	302	
7.07	3.3	24	2.5	2.9	664	
"	5.9	48*	"	4.0	434	
6.42†	3.6	33*	1.5	3.0	270	
"	5.4	51*	"	3.9	509	
6.0†	3.3	46	1.0	2.8	1425	
"	4.5	43	"	4.0	±1140	
5.85†	3.5	61	0.5	2.3	510	
"	4.4	42*	"	2.9	665	
5.3	3.0	59	0.25	2.3	734	
"	4.3	61*	"	4.1	419	
4.85	2.5	58*				
"	5.4	66*				
9.0	2.8	40	1.5	3.1	389	Boiling point 105° to to 107° C.
"	3.3	40	"	4.7	703	
6.0	3.3	42	1.0	3.8	783	
"	3.3	42	"	4.5	663	
4.0	2.8	77	0.5	4.0	390	
"	3.7	±87	"	4.2	664	
2.52	3.1	282	0.25	3.8	784	
"	4.2	262*	"	4.0	±1200	

TABLE XXI

PHENOL. TEMPERATURE 21.5°C.

g. per liter	Weight of fish in grams	Survival time of fish in minutes	g. per liter	Weight of fish in grams	Survival time of fish in minutes	Remarks
3.45	3.2	9	0.345†	2.4	59	
"	3.7	9	"	2.6	103	
2.58	3.3	10	0.31†	2.9	81	
"	3.9	10	"	3.4	53	
1.72	2.4	13	0.26†	2.55	104	
"	3.1	13	"	2.8	132	
1.036	2.5	17	0.17	3.2	125	Daggers (†) as in Table
"	3.7	24	"	3.5	136	I.
0.69	2.8	18	0.10	3.2	91	
"	3.8	30	"	2.9	77	
0.517	2.3	32	0.07	3.3	292	
"	2.5	38	"	3.7	217	
0.414†	2.0	37	0.051	?	92	
"	3.4	51	"	3.4	140	

TABLE XXII

CAFFEINE. TEMPERATURE 17°C.

g. per liter	Weight of fish in grams	Survival time of fish in minutes	g. per liter	Weight of fish in grams	Survival time of fish in minutes	Remarks
2.22	2.4	13	0.32†	2.8	22	
"	2.9	10	"	3.5	83	
1.58	3.0	14	0.284†	3.4	94	
"	3.0	18	"	3.8	256	
0.95	3.0	22	0.19	2.4	319	
"	3.2	23	"	2.4	359	Daggers (†) as in Table
0.712	2.6	23	0.095	2.8	322	I.
"	2.9	25	"	3.0	1140	
0.47	2.8	23	0.047	2.8	1200	
"	3.0	50	"	3.1	1652	
0.40†	2.2	42	0.019	3.0	1320	
"	3.2	45	"	3.5	2760	

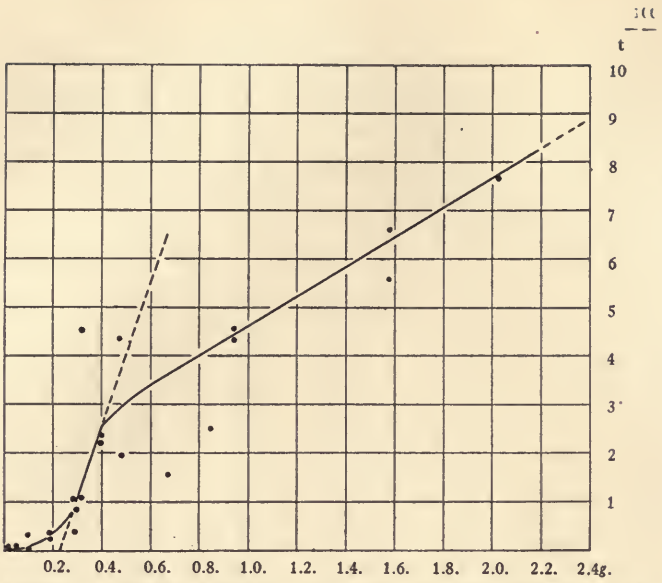

Figure 18. Caffeine. Velocity of fatality curve only. Abscissa represents g. per liter

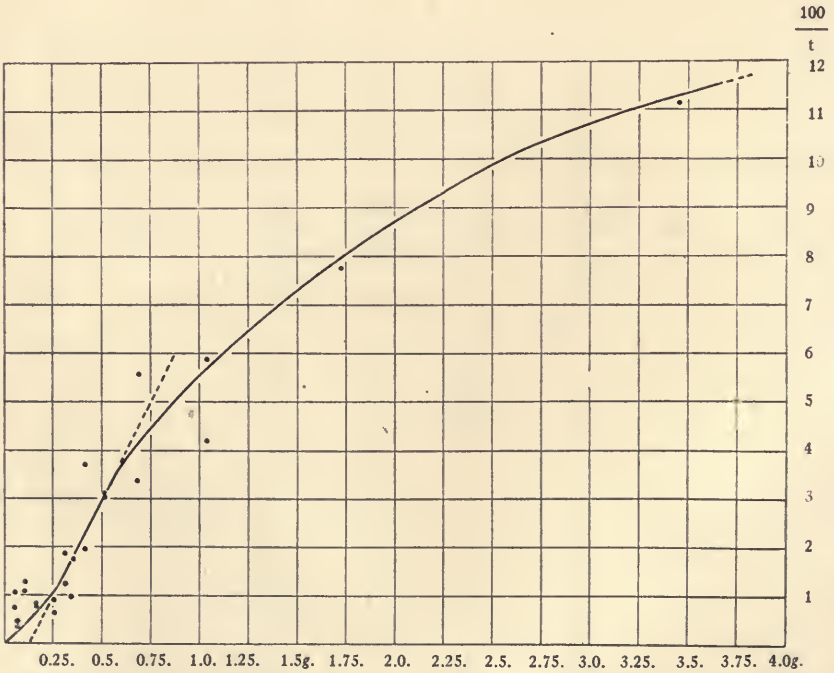

Figure 19. Phenol. Velocity of fatality curve only. Abscissa represents g. per liter.

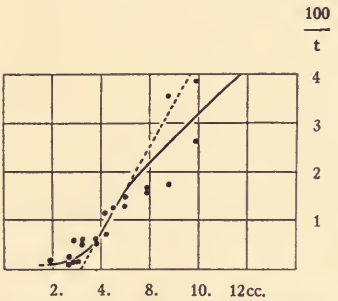

Figure 20. Pyridine. Velocity of fatality curve only. Abscissa represents cc. per liter

TABLE XXIII

PYRIDINE. TEMPERATURE 17.5°C.

cc. per liter	Weight of fish in grams	Survival time of fish in minutes	cc. per liter	Weight of fish in grams	Survival time of fish in minutes	Remarks
7.87	2.0	26*	3.0	2.7	±720	
"	2.6	38	"	3.1	±720	
6.75	2.4	28	2.81	3.5	180	
"	3.6	58	"	3.7	±900	
5.81	3.3	63	2.62	3.3	420	Daggers (†) as in Table I.
"	3.8	61	"	4.0	±1080	
4.87	2.1	68	1.87	2.4	715	*Fish was not dead when taken out of solution. *1Temperature 21.5 C. Fish were suffering with gas disease.
"	2.9	78	"	2.9	610	
4.5†	2.2	82	10.0*1	3.1	13*	
"	4.1	83	" *1	3.1	15*	
4.12†	2.5	87	5.0*1	2.2	40	
"	2.7	140	" *1	2.5	40	
3.75†	2.2	175	2.0*1	3.3	±1020	
"	3.1	187	" *1	3.3	660*	
3.19†	2.2	180				
"	2.2	190				

TABLE XXIV

The maximum and minimum concentration of substance and maximum and minimum survival time of goldfish within the range in which the velocity of fatality curve approaches a straight line.

Substance	Normal	Survival time of fish in minutes
$NaCl$	0.500	33
"	"	34
"	0.277	178
"	"	260
$LiCl$	0.322	54
"	"	58
"	0.166	234
"	"	310
KCl	0.328	22
"	"	26
"	0.214	69
"	"	150
NH_4Cl	0.168	38
"	"	37
"	0.224	99
"	"	90
$NaNO_3$	0.380	122
"	"	109
"	0.220	171
"	"	196
NH_4NO_3	0.253	37
"	"	52
"	0.213	78
"	"	82
$MgCl_2$	0.432	23
"	"	30
"	"	34
"	0.313	61
"	"	88
"	"	80
$CaCl_2$	0.356	48
"	"	59
"	0.249	174
"	"	190
$SrCl_2$	0.318	59
"	"	76
"	0.237	168
"	"	347
$BaCl_2$	0.249	43
"	"	80
"	0.172	169
"	"	316
$Mg(NO_3)_2$	0.370	37
"	"	56
"	0.229	107
"	"	104
$Ca(NO_3)_2$	0.281	45
"	"	50
"	0.192	186
"	"	186
$Sr(NO_3)_2$	0.192	92
"	"	87
"	0.165	300
"	"	600
KCN	0.25×10^{-4}	146
"	"	204
"	0.2×10^{-4}	2135
"	"	1945
HCl	8.99×10^{-4}	46
"	"	72
"	0.313×10^{-4}	1200
"	"	2520
g. per L.		
Phenol	0.414	37
"	"	51
"	0.259	104
"	"	132
Caffeine	0.396	42
"	"	45
"	0.285	256
"	"	94
cc. per L.		
Methyl Alcohol	42.5	52
"	"	58
"	25.0	206
"	"	326
Ethyl Alcohol	51.5	26
"	"	28
"	16.0	98
"	"	88
Iso-butyl Alcohol	6.42	33
"	"	51
"	5.85	61
"	"	42
Pyridine	4.5	82
"	"	83
"	3.187	180
"	"	190

TOXICITY OF CUPRIC CHLORIDE, CADMIUM CHLORIDE, FERRIC CHLORIDE, COLLOIDAL COPPER, AND DISTILLED WATER

It has already been pointed out that the toxic activity of various concentrations of $CuCl_2$, $CdCl_2$ and $FeCl_3$ did not follow the same general law as did all other substances tested. The toxicity of the $CuCl_2$ at very high concen-

TABLE XXV

CUPRIC CHLORIDE. TEMPERATURE 21°C.

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
0.66	2.0	78	5.7×10^{-3}	3.2	62*	
"	2.7	85	"	3.2	137	
0.58	2.6	83	4.5×10^{-3}	2.5	58	
"	2.7	84	"	2.6	53	
0.43	3.0	85	2.6×10^{-3}	3.5	117	
"	3.1	85	"	5.0	128*	
0.33	2.0	45*	2.8×10^{-3}	2.8	59	
"	2.2	39	"	2.8	69	
0.25	2.2	38	2.3×10^{-3}	2.8	83	
"	3.0	30	"	3.3	80	
0.19	1.8	44*	1.8×10^{-3}	3.3	117	
"	1.95	49*	"	3.5	100	
0.15	2.8	92	1.2×10^{-3}	3.2	90	
"	3.2	52*	"	4.3	97	
0.11	2.7	96	9.1×10^{-4}	3.2	100	*Fish was alive when taken out of solution.
"	3.0	70	"	3.5	140	
0.068	1.9	85	4.5×10^{-4}	2.9	120*	
"	1.9	87	"	2.9	136	
0.034	1.7	133	2.2×10^{-4}	3.8	112	
"	1.9	118	"	4.1	116	
0.023	3.5	44*	1.1×10^{-4}	3.7	158*	
"	4.0	56*	"	3.8	112	
0.017	2.1	60*	0.56×10^{-4}	4.1	125	
"	2.3	66	"	4.2	260	
"	2.5	80	0.19×10^{-4}	3.0	214	
"	3.8	63	"	3.3	±300	
0.011	2.8	52*	0.13×10^{-4}	3.5	±315	
"	3.2	51*	"	3.9	367	
9.1×10^{-3}	2.5	146	0.34×10^{-5}	3.9	117*	
"	2.5	164	"	3.9	208	
7.9×10^{-3}	2.9	115*	0.11×10^{-5}	2.8	±300	
"	3.2	59*	"	3.9	403	
5.7×10^{-3}	3.4	70	0.28×10^{-5}	3.1	216	
"	3.5	70	"	4.4	430	

TABLE XXVI

CADMIUM CHLORIDE. TEMPERATURE 21.5°C.

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
0.157	1.95	68	0.92×10^{-3}	3.2	167	
"	2.0	70	"	4.5	177	
0.1191	1.6	59*	0.42×10^{-3}	3.0	169	
"	2.0	61	"	4.3	204	
0.1036	1.7	61	0.24×10^{-3}	3.8	272	
"	2.3	62	"	4.0	208	
0.0903	1.65	59	0.12×10^{-3}	3.0	163	
"	1.75	39	"	4.0	165	
0.078	1.9	43	6.6×10^{-5}	2.8	148	
"	2.0	42	"	2.9	189	
0.065	1.9	54	3.7×10^{-5}	2.7	215	
"	2.0	57	"	4.1	292*	
0.057	1.9	59	1.8×10^{-5}	3.0	325	
"	1.9	60	"	4.1	126	
0.048	1.7	75	1.1×10^{-5}	4.4	349	
"	2.1	77	"	4.5	400	
0.043	1.7	73	0.74×10^{-5}	3.0	248	
"	1.9	67	"	3.0	335	*Fish was not dead when taken out of solution.
0.037	1.5	41	0.37×10^{-5}	3.9	360	
"	1.9	82	"	4.0	349	
0.032	1.65	78	"	3.6	199	
"	1.7	81	"	3.6	224	
0.024	1.8	120	0.18×10^{-5}	4.0	311	
"	2.0	121	"	4.7	261	
0.021	1.6	93	"	2.9	237	
"	1.75	100	"	3.8	349	
0.018	1.9	102	9.2×10^{-7}	3.8	335	
"	2.7	118	"	4.3	373	
0.015	1.6	164	5.2×10^{-7}	4.1	286	
"	2.15	136	"	4.6	316	
6.6×10^{-3}	2.0	132	2.6×10^{-7}	3.6	445	
"	2.9	130	"	4.6	353	
"	3.7	206	1.3×10^{-7}	3.4	635	
"	3.7	216	"	3.7	635	
3.7×10^{-3}	1.9	115	0.74×10^{-7}	4.7	861	
"	2.0	135*	"	5.5	±960	
"	3.4	233	0.37×10^{-7}	3.7	442	
"	3.6	295	"	3.8	479	
1.8×10^{-3}	3.4	193*	0.18×10^{-7}	3.9	±1080	
"	3.7	206	"	4.3	523	

trations (0.66 to 0.25 N. See Table XXV) increased with decrease in concentration. The shortest survival time of any goldfish was 30 minutes. From this point (0.25 N.) the toxicity of the $CuCl_2$ decreased with decrease in concentration but the decrease was very slight as compared to the dilution until the maximum survival time of the goldfish was 430 minutes. With the $CdCl_2$ there was also an increase in toxicity with a decrease in concentration with the highest concentrations tested (0.157 to 0.078 N. See Table XXVI). The two goldfish died in 42 and 43 minutes in 0.078 N. $CdCl_2$. From this point

TABLE XXVII

FERRIC CHLORIDE. TEMPERATURE 21.5° C.

Normal	Weight of fish in grams	Survival time of fish in minutes	Normal	Weight of fish in grams	Survival time of fish in minutes	Remarks
0.284	2.1	28*	8.32×10^{-3}	1.8	89	
"	2.3	29	"	1.9	109	
0.213	1.5	26*	"	3.3	247	
"	1.7	28	"	3.6	115	
0.159	1.7	26*	3.88×10^{-3}	2.8	101	
"	2.2	31	"	3.2	127	
0.119	1.5	38*	"	2.1	121	
"	1.8	32	"	2.1	129	*Fish was not dead when taken out of solution.
0.0693	1.7	33*	1.94×10^{-3}	3.0	131	
"	3.0	43*	"	3.4	113	
0.0554	1.9	46*	1.1×10^{-3}	3.4	164	
"	2.1	38	"	3.6	180	
0.0332	1.8	56	5.5×10^{-4}	3.3	450	
"	2.2	59	"	3.9	723	
0.0166	1.7	84	2.8×10^{-4}	2.7	790	
"	2.1	74	"	3.4	±1080	
"	3.3	89	1.66×10^{-4}	3.0	±1200	
"	3.7	83	"	3.2	±1200	

the toxicity decreased very slowly in proportion to the dilution of the solution until the maximum survival time of the goldfish was 1080 minutes in 0.18×10^{-7} N. The toxicity of the $FeCl_3$, unlike $CuCl_2$ and $CdCl_2$, decreased progressively with the dilution of the solution from the highest concentration (0.284 N. See Table XXVII) to the lowest concentration (0.166×10^{-3} N.) tested when the survival time of the goldfish reached a maximum of 1200 minutes.

The variations of the toxicities of these three salts from that of other substances tested can possibly be explained by the fact that they form colloidal

solutions and that colloids are very toxic and that the amount of colloid in the solution is not in proportion to the concentration of the salt in solution. Miyake (1916) found 1/75000 N. $AlCl_3$ was toxic to the growth of rice seedlings which he attributed to colloidal $AlCl_3$. Robin (1914) showed that the toxic activity of colloidal sulphur was much more intense than that of other forms of sulphur. The maximum survival time of the goldfish in ordinary distilled water was about the same as that in the most dilute $CuCl_2$ tested. Mengarini and Scala have shown that numerous metals, like copper and aluminum, form colloidal solutions in distilled water at room temperature. The toxicity of the distilled water, since it was distilled in copper stills with block-tin leads, was due possibly to the colloidal $CuO \times H_2O$ and $SnO_2 \times H_2O$. Ringer (1883) found that goldfish would live from 46 to 54 hours in distilled water. Bullot (1904) found that the survival time of Gammarus was shorter in water

TABLE XXVIII
WATER DISTILLED IN A COPPER STILL WITH BLOCK-TIN LEADS
TEMPERATURE 21.5° C.

Weight of fish in grams	Survival time of fish in minutes
3.4	352
3.8	512
3.9	597
4.3	367

TABLE XXIX
WATER DISTILLED IN GLASS

Weight of fish in grams	Survival time in days
2.7	41
?	51
?	30
3.2	37

TABLE XXX
COLLOIDAL COPPER
TEMPERATURE 21.5° C.

Exp.	Weight of fish in grams	Survival time of fish in minutes
1	4.3	900
1	4.6	900
2	4.0	2880
2	4.9	2820

distilled in copper than in ordinary glass, Jena glass, quartz glass, and platinum. The latter were all toxic to about the same extent.

Three sets of experiments were run to test the supposition that a colloidal solution was responsible for the short survival time of the goldfish in ordinary distilled water. 1. The goldfish lived 36 days in distilled water after an electrolyte (0.025 N. NaCl) had been added to precipitate the colloid. 2. The goldfish lived 30 to 52 days in water distilled in ordinary glass. 3. A colloidal solution of copper was prepared in water distilled in ordinary glass by arcing copper wires under the surface of the water. To one quantity of water a smaller amount of colloidal copper was added and the goldfish lived about 2820 minutes. To another a larger amount of colloidal copper was added and the goldfish lived only about 900 minutes. Thus the greater toxic

activity of water distilled in copper stills with block-tin leads and possibly also the $CuCl_2$, $CdCl_2$, and $FeCl_3$ solutions is probably due to the presence of a colloid. This is in keeping with Locke's (1895) results in which he showed that distilled water may lose its toxic activity by long boiling, and especially when brought in contact with sulphur, carbon, manganic oxide, cotton, wool, silk, and other substances, and those of Wells (1915) who found distilled water no more toxic than tap-water so long as the distilled water was slightly acid.

THE EFFICIENCY OF THE GOLDFISH AS A TEST ANIMAL

In all substances tested the goldfish died fairly uniformly in any given concentration where the survival time did not exceed five hours. See Tables I to XXIV for data of experiments in which two goldfish were killed in each solution tested. The variation of the survival time is better shown by data of experiments given in Tables XXXI and XXXII in which a number of fish were killed in each solution. The broken line in Figure 21 is a graphic representation of the survival times of the 8 goldfish killed in 0.272 N. LiCl solu-

TABLE XXXI

THE VARIATION IN THE SURVIVAL TIME OF THE GOLDFISH. TEMPERATURE 20° TO 21° C.

0.272 N. Lithium Chloride		0.229 N. Ammonium Chloride		0.222 N. Ammonium Chloride		0.231 N. Potassium Chloride	
Weight of fish in grams	Survival time of fish in minutes	Weight of fish in grams	Survival time of fish in minutes	Weight of fish in grams	Survival time of fish in minutes	Weight of fish in grams	Survival time of fish in minutes
1.9	79	2.3	49.5	2.5	51	1.5	17
2.0	78	2.4	49.5	2.6	62	2.3	51
2.2	88	2.6	52	2.65	71	2.5	49
2.2	89*	2.6	74	2.7	51	2.5	43
2.25	87	2.8	53	2.9	78	2.55	69
2.3	81	2.9	54	2.9	85	2.6	69
2.4	70	2.0	88	2.9	126	2.8	47*
2.4	86	3.0	69	3.0	69	2.8	48
		3.1	104				

*Fish was not dead when taken out of solution

tion, Table XXXI. One block of abscissa represents an individual goldfish and one block ordinate represents 100 minutes survival time of the goldfish in the 0.272 N. LiCl. The goldfish with the shortest survival time is represented first and the second next and so on to the goldfish with the longest survival time. Thus the deviation of the line from the horizontal represents the variation of the survival time of the goldfish in the given solution. Each of the other lines of the graphs in Figures 21 and 22 represents the survival times of individual goldfish in a definite concentration of a particular substance tested. In all graphs one block ordinate represents 100 minutes survival time and one block abscissa represents an individual goldfish. In these

graphs the weight of the goldfish is not taken into consideration. The goldfish with the shortest survival time is always considered first, the second next, and so on. Small variations in the weight of the goldfish had very little effect on their survival time, though in general the smaller died first. Of all goldfish killed the smaller died first in the ratio of 2.1 to 1. Where there was a greater variation in size the smaller died first in a much greater proportion. For variations of the survival time of the goldfish as compared to their relative weights see Tables I to XXXVI. In all the tables the smallest goldfish is placed first, the next smallest second, and so on.

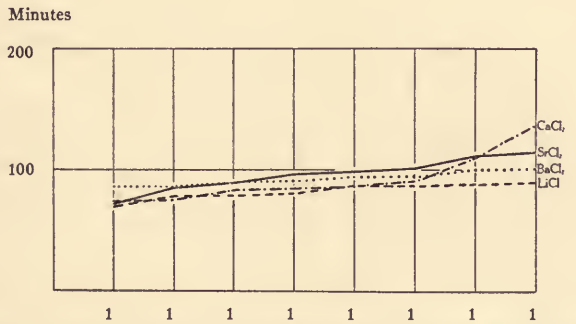

Figure 21. Graph showing the efficiency of the goldfish as a test animal. Ordinate represents the survival time in minutes and abscissa represents individual fish

Dot and dash line = 0.284 N. $CaCl_2$
Solid line = 0.274 N. $SrCl_2$
Dotted line = 0.214 $BaCl_2$
Broken line = 0.272 N. LiCl

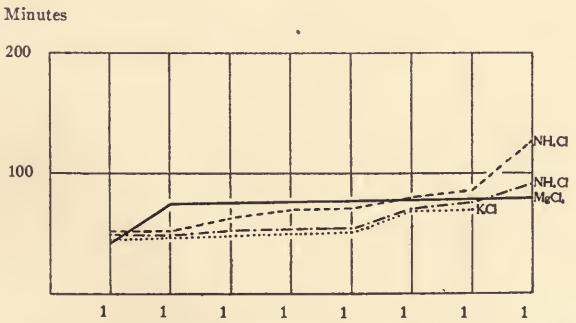

Figure 22. Graph showing the efficiency of the goldfish as a test animal

Broken line = 0.222 N. NH_4Cl
Dot and dash line = 0.229 N. NH_4Cl
Solid line = 0.318 N. $MgCl_2$
Dotted line = 0.231 N. KCl

THE EFFECT OF TEMPERATURE UPON THE RESISTANCE OF GOLDFISH

A few experiments were run to determine the effect of temperature on the toxic activity of a substance on the goldfish. It is shown by Tables XXXIII, XXXIV, and XXXV that the survival time of the goldfish in a constant concentration of a substance is lowered by a rise in temperature. This agrees with other workers on toxicity. See bibliography for citations. No attempt was made to find the relation between temperature and the toxic activity of a substance. See discussion of Warren's (1900) temperature toxicity curve, page 52.

TOXICITY AND THE EXPRESSION OF RELATIVE TOXICITY

The relative toxicity of the respective elements has long interested the chemist. The views of workers on the toxic activity of the elements have varied from time to time to fit any newly discovered physico-chemical conception of the properties of the elements. James Blake (1883, 1887) associated the physiological action of the elements of the isomorphous groups with their atomic weights. Botkin (1885) suggested a relation between the toxic activity of the elements and their position in the periodic system. Pauli (1903), Kahlenberg and True (1896), Kahlenberg and Austin (1900), Loeb (1902), and Mathews (1903, 1907) held that the toxic activity of a substance is dependent either upon the free electrical charge or the atom itself while in the atomic state. Richet (1881) and Rabuteau have suggested that toxicity is a function of the solubility of the substance. Mathews (1904) has related toxicity to the solution tension of the ion. While, finally, Bert (1871), Garrey (1905), Křiženecký (1916) and others claim that the toxic effect of at least certain elements to fresh water animals is due primarily to osmotic pressure. After all these and other suggestions such as coagulation of the protoplasm, ionic combination, either physical or chemical, with the protein of the protoplasm, and the change of permeability of the cell membrane, the cause of toxicity is still an open question. Aside from the divergence of opinions as to the cause of the active properties of the elements there has been no absolute agreement on relative toxicity itself. In this work some attempt has been made to determine the relative toxic values of certain of the alkali and alkaline earth metals when in combination with Cl and NO_3. First it is obvious that it is necessary to have some standard of measure of the elements themselves. The elements will be arranged differently from the same data when considered by actual weight of the element, actual weight of the salt, molecular concentration, and normality. This of itself explains certain of the disagreements among workers, though after all the elements are reduced to a common standard of measure there is still a wide divergence in relative toxicity as reported by different workers. Osterhout (1915) has pointed out that "the relative toxicity of two substances may depend very largely on the stage of the reaction at which the measurement is made"; i.e., the criterion employed. Osterhout objects

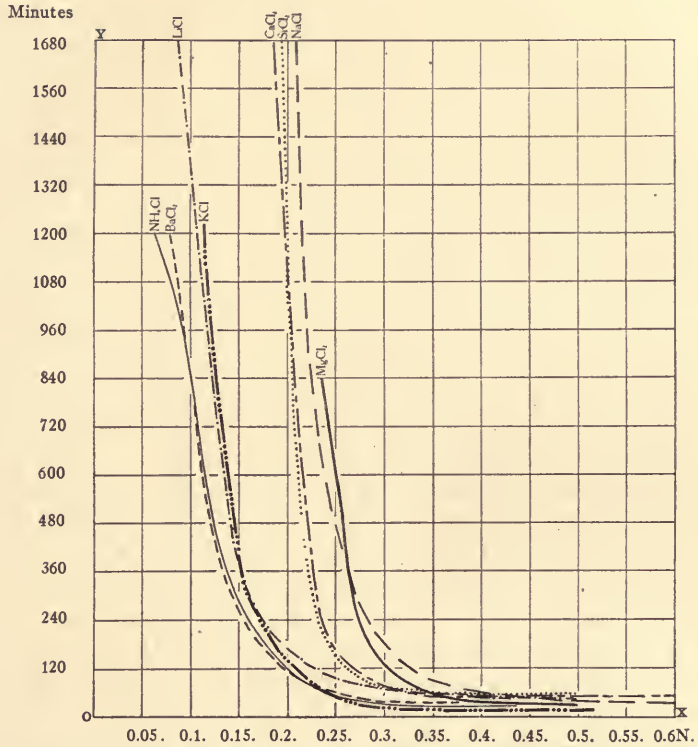

Figure 23. Superimposition of survival time curves of the alkali and alkaline earth metals as chlorides showing crossing over of curves, thus showing varying relative toxicities of the group when different definite survival periods are taken as a criterion.

to the utilization of the death point as a criterion and points out the fact that "it is impossible to determine the precise moment of death." He has shown that the death curve "approaches the axis asymptotically." He suggests that it may be assumed that as the reaction proceeds certain phenomena appear at definite points on the curve. He took as his criterion a point on the curve at which he said that the organism was "half dead." The writer has found the same objections pointed out by Osterhout but has come to the conclusion that the death point with certain precautions is a more exact criterion for an end point in the case of the goldfish than the loss of equilibrium or irritability. Aside from the objections raised by Osterhout there is a still more serious objection to any attempt to determine relative toxicities by comparing the concentrations of the solutions or the amount of the substance necessary to produce death or the appearance of any physiological phenomenon in any arbitrary fixed time or the concentration necessary to just cause

death. The relative toxic activities of the substances will vary according to the criterion used. Only a glance at figure 23 is required to convince one that there will be a marked rearrangement in the relative toxic values of the elements when different survival times are used as criteria. For example, if a 120 minute survival time is chosen as a criterion the relative toxicities of the elements as chlorides with normality as the standard of measure will be arranged from the most toxic to the least toxic in the following order:—Ba, K, Li, Sr, Ca, Mg, and Na, and with a 60 minute survival time they will be arranged as follows:—K, Ba, Ca, Li, Sr, Mg, and Na. Thus there is a marked rearrangement of the relative positions of the elements with a change from a 120 minute survival time as a criterion to that of a 60 minute survival time. It is the opinion of the writer that it is the employment of different criteria that is responsible more than any other one thing for the differences of opinions of workers on the toxic values of the elements. The fact is not denied that the elements may have different relative activities toward different organs of the same animal as well as toward different animals (Kahlenberg and Mehl 1901). By a study of the survival time curves shown in Figure 23 it is seen that the activity of each element is a law unto itself, and thus there is no one expression that will include all specificities of the salts or toxic substances. But a standard criterion may be derived. From a study of the velocity of fatality curves it is seen that there are two variables and that these vary independently of each other. 1. The concentration necessary to just kill the goldfish varies with the element. 2. The acceleration of the change of direction of the survival time curve, i.e., the increase in the velocity of fatality is different in each of the salts and does not necessarily have any relation to the amount of the salt necessary to just kill the goldfish. The value of the first factor is shown by the position of the point C and the second by the slant of the velocity of fatality curve. But since the slant of the velocity of fatality curve is not uniform for all concentrations of a substance the theoretical velocity of fatality curve will be considered. This curve bears the same relation to the physiological activity of the substance as the true velocity of fatality curve. Thus the relative toxicities of substances due to the first factor can be measured by the reciprocal of a, the distance P from the origin O. That due to the second can be measured by the tangent of the angle XPB, Θ. (See Figure 1.) Both these factors must be taken into consideration in a criterion or an expression representing the relative toxicity of a substance. The tangent Θ increases as the toxicity increases with respect to the slope of the theoretical velocity of fatality curve, i.e., the rapidity with which the activity of the substance increases with increase in concentration and the distance of the point P from the origin O or a decreases as the toxicity increases with respect to the theoretical threshold of toxicity concentration, i.e., as the theoretical threshold of toxicity concentration decreases. Thus it is seen that these two factors are the reciprocal of each other. This relation can be expressed mathematically by the equation,

T (toxicity) $= \sqrt{\frac{\text{tan. } \theta}{a}}$ This equation cannot represent the absolute or the exact relative toxic value of a substance since it is based only on a portion of the velocity of fatality curve, the portion A to B, Figure 1, and the assumption that this portion and the two extremes, i.e., the two extremes C to A and B to G, follow the reciprocal of an equilateral hyperbola which is not in keeping with experimental data or a curve drawn from the hypothetical equation representing the true velocity of fatality curve. See pages 48, 52 for a discussion of this hypothetical equation. However this expression has been chosen to represent the relative toxic value of a substance rather than the curve itself as suggested by Osterhout (1915), as the latter leaves the formation of a criterion to the reader. It has the advantage in that the relative toxicity can be given a numerical value and is a natural criterion and not an arbitrary one. This equation, as has already been pointed out, does not express the specificity of the toxic activity of the salt, which can be shown only by the curve itself, as Osterhout has suggested, or by taking into consideration the factors which go to make up an equation which represents the curve itself (See hypothetical equation page 49). Taking the above equation as representing the relative toxicities of the alkali and alkaline earth metals, as chlorides and nitrates, the toxic values are found as given in Table XXXVI. These values are comparable in general, not so much in comparative numerical values but in position, to values obtained by other workers after the measurements of the elements are reduced to a common standard. See bibliography for citations. However, among all workers there is more or less difference of opinion.

TABLE XXXVI

Relative Toxicities of the Alkali and Alkaline Earth Metals when in Combination with Cl and NO_3

Substance	Relative toxicity
NaCl	1.24
LiCl	1.53
KCl	2.3
NH_4Cl	2.87
$CaCl_2$	1.56
$SrCl_2$	1.57
$MgCl_2$	1.87
$BaCl_2$	1.94
$NaNO_3$	1.00
NH_4NO_3	1.95
$Mg(NO_3)_2$	1.75
$Ca(NO_3)_2$	1.92
$Sr(NO_3)_2$	2.58

IS THE TOXICITY OF A SUBSTANCE DUE TO OSMOTIC PRESSURE?

A series of experiments was run with d-glucose (Table XXXVII) to determine the effect of osmotic pressure on the goldfish. When the molecular concentration of the glucose is divided by two to make its osmotic pressure comparable to that of a normal salt solution, it is seen that the toxicity of the glucose is still below that of the particular salt to which it is compared. This difference is even more striking when the fact that the salts are not completely ionized is taken into consideration and that this is more than sufficient to allow for complete ionization of salts of bi and tri valent acids and bases. The improbability that the toxicity of a substance is due to osmotic pressure is further emphasized by the fact that the toxic activities of substances like potassium cyanide, hydrochloric acid, the alcohols, caffeine, phenol, and pyridine are not in proportion to their molecular concentrations.

TABLE XXXVII

D-GLUCOSE. TEMPERATURE 21.5°C.

Mole	Weight of fish in grams	Survival time of fish in minutes	Mole	Weight of fish in grams	Survival time of fish in minutes
1.	2.3	158	0.15	2.4	5700
"	2.7	77	"	2.3	7140
0.6	2.5	311	0.075	3.0	5640
"	3.8	356	"	3.3	7860
0.4	2.7	±1200	0.05	2.8	2880
"	3.2	±2640	"	4.7	4560
0.2	2.2	±8400	0.025	2.8	2880
"	2.9	±8400			

THEORY OF THE POISONING EFFECT ON TOXIC SUBSTANCES

Osterhout (1915) has shown in a very interesting paper that death in the Laminaria, when placed in NaCl solution of the same conductivity as that of sea-water, as measured by the fall of electrical resistance, does not follow a straight line when electrical resistance is plotted as ordinate and time as abscissa, but follows a curve which, he points out, "follows the course of a monomolecular reaction."

The effect of poison on protoplasm might be compared to a system of chemical reactions which finally result in the death of the organism. It has been shown outside the living organism that KCN retards oxidation. Kastle

and Loevenhart (1901) pointed out the fact that the activity of oxidase of the potato was retarded by KCN. Shafer (1915) found that hydrocyanic acid affects catalases, reductases, and oxidases in insect tissues. Mathews and Walker (1909) showed that very small amounts of KCN is sufficient to check or prevent the spontaneous oxidation of cysteine to cystin both in neutral and alkaline solutions. Loevenhart and Kastle (1903) have shown that hydrocyanic acid inhibits the catalytic activity of solutions of metallic colloids. Claud Bernard (1857) noted that venous blood of an animal poisoned with hydrocyanic acid takes on an arterial hue. Geppert (1889) showed venous blood to have a higher oxygen content than normal in mammals poisoned with hydrocyanic acid. Richards and Wallace (1908) found that protein metabolism is increased in a dog by poisoning with KCN due in part to the retarding effect on oxidation by the KCN. Loeb and others have shown that oxygen requirement is decreased and that this decrease is greater the greater amount of KCN used. All have shown that KCN in very small amounts acts as a stimulus to oxidation both in and out of the animal organism. Moore and Moore (1917) have shown that the maturity of fruit was two weeks earlier and that the total yield of fruit was larger on tomato plants fumigated with HCN than normal plants. Woodworth (1915) showed that scale insects eggs when fumigated with hydrocyanic acid in amounts not sufficient to kill them hatched earlier than the normal eggs. Townsend (1901) has found that there is an increase in germination in seed fumigated with hydrocyanic acid. And Zieger (1915) in turn has shown that the activity of the catalase is in proportion to the general metabolic activities in animals. While Child (1915, p. 66 and citations) and others have shown that very small amounts of KCN increase the rate of metabolism while larger amounts decrease metabolic processes, Mathews (1916, p. 813) suggests that since cysteine oxidizes itself and has many points in its oxidation which resemble respiration there is more than a superficial connection between the oxidation of cysteine and the respiration of the cell. The most favorable hydrogen ion concentration for oxidation is the same as that of protoplasm and both cysteine and protoplasm are poisoned by many of the same substances, as nitriles, the cyanides, acids, and heavy metals. Their oxidations are catalyzed or hastened in the same manner by iron, arsenic, and certain other agents. Burge and Burge (1914, 1915) have shown that digestion of a dead round worm in activated pancreatic juice does not take place so long as its body wall is constantly permeated with nascent oxygen. They conclude that the oxidative processes of living parasites enable them to withstand the enzymes by oxidizing the enzymes in contact with them. Lillie (1902) advances the theory that the tissues are protected from autolysis by oxidizing the enzymes in contact with them. Hofmeister (Mathews 1916, p. 83), Verworn (1909, p. 53), Hopkins (1914), and others have held that there is no vital matter in the cell and that the chemical transformations do not involve any large biogene molecule but only relatively simple compounds in solution. But Pflüger, Du Bois-Reymond (Mathews,

1916, pp. 842-844), Driesch (1909), and Sir Oliver Lodge (1913) suggest that there is an organized force, entelechy, or vital force and that so long as the cell is alive this force regulates the metabolism but as soon as this force is destroyed or inhibited the metabolic processes cease, i.e., the machinery no longer works and the cell dies. Robertson (1908) suggests that the normal rate of growth is an autocatalytic process. And finally Troland (1917) defends the thesis that all biological enigmas as he calls them are explainable on the basis of enzymes either autocatalytic or heterocatalytic. Thus it appears from either the mechanistic or the vitalistic view and experimental data that metabolism can be conceived of as a self-perpetuating mechanism and that when metabolism is interfered with beyond a certain limit, this mechanism or metabolism is progressively depressed or inhibited. That is, metabolism continues within a certain limit at a normal rate; enzymes, excitors, or antibodies inhibiting autolysis are liberated or generated which stimulate metabolism or allow metabolic activities to continue at a normal rate. But as rapidly as the rate of metabolism is reduced below this normal rate, beyond a certain limit, the rate of liberating or generating enzymes or antibodies is reduced in proportion to the reduction of the rate of liberation or generation of the cell enzymes or antibodies. In other words, we are dealing here with the rate of inhibition of a process, metabolism, the rate of which is uniform under any fixed set of normal conditions. One of the factors of a normal condition is a normal rate of metabolism. Thus when the rate of metabolism is reduced below the normal the conditions become abnormal. This in turn becomes an inhibiting factor which increases progressively with the decrease in the rate of metabolism, i.e., it becomes self-inhibiting. This portion of the effect on the speed of inhibition of metabolism is thus proportional to the product of the amount of reduction in the rate of metabolism at any time t, and the reduced rate of metabolism at that time. This can be compared in the speed of the reduction of the rate of metabolism to the velocity of an autocatalytic reaction and can thus be expressed mathematically $\frac{dz}{dt} = K_2 z(M-z)$. That is, at any given time, t, the speed of inhibition of metabolism is in proportion to the product of the reduced rate of metabolism and the rate of metabolism at the given time. M = normal rate of metabolism under any fixed set of conditions, z = amount of reduction of rate of metabolism at any given time, K_2 = a constant representing the efficiency of the reduced rate of metabolism in inhibiting metabolic processes, i.e., in inhibiting the activation or action of the metabolic enzymes or antibodies or representing the liberation of autolytic enzymes, and t = time. The amount of reduction z in the rate of metabolism after any time t is due not only to the autoinhibitory process but also to the continuous action of the protoplasmic poison introduced. But the equation above takes into consideration only the reduction due to the autoinhibitory process, and does not take into

consideration the continuous action of the poison on the rate of reduction of metabolism. But at any given time the speed of reduction of the rate of metabolism due to the continuous action of the protoplasmic poison is in proportion to the amount of poison acting at the given time and the actual rate of metabolism, i.e., (M-z). Thus the speed of the inhibition of the rate of metabolism by the continuous action of the protoplasmic poison can be compared to the velocity of a simple mass action. And since the fish is in a solution the volume of which is large as compared to the volume of the fish and since the poison is continuously diffusing into the protoplasm to the point of equilibrium, the amount of protoplasmic poison can be considered as constant throughout the entire process. The equation representing the speed of reduction of rate of metabolism by the continuous action of the protoplasmic poison then becomes $\frac{dz}{dt} = K_1X(M\text{-}z)$. K_1 = the efficiency of the protoplasmic poison in reducing the rate of metabolism and X = the amount of poison employed and is a constant for any definite amount of poison employed and appears in the equation only for the sake of comparing the action of different amounts of protoplasmic poison employed in any two experiments.

Since both of the processes indicated by the above equations are acting simultaneously to reduce the rate of metabolism, the actual rate of reduction is the sum of the two left members. If we now denote this actual rate of reduction by $\frac{dz}{dt'}$, then $\frac{dz}{dt} = K_1X(M\text{-}z) + K_2z(M\text{-}z)$.

Integrating we have t+C (constant of integration) = $\frac{1}{MK_2+K_1X} \log_e \frac{K_1X+K_2z}{K_2(M\text{-}z)}$

But when t=0, z=0. Then $C = \frac{1}{MK_2+K_1X} \log_e \frac{K_1X}{K_2M}$.

By substituting the value of C and transforming we have:

$$t = \frac{1}{MK_2+K_1X} \log_e \frac{K_1X+K_2z}{K_2(M\text{-}z)} - \frac{1}{MK_2+K_1X} \log_e \frac{K_1X}{K_2M} =$$

$$\frac{1}{MK_2+K_1X} \log_e \left(\frac{M}{M\text{-}z} + \frac{K_2Mz}{K_1(M\text{-}z)} \cdot \frac{1}{X} \right).$$

$$\text{Or } \frac{1}{t} = Y = \frac{MK_2+K_1X}{\log_e \left(\frac{M}{M\text{-}z} + \frac{K_2Mz}{K_1(M\text{-}z)} \cdot \frac{1}{X} \right)}$$

The reciprocal of the survival time of the goldfish $\frac{1}{t}$ is represented as ordinate in the graphs of the velocity of fatality curves, thus $\frac{1}{t} = Y$.

Any mathematical expression of the velocity of fatality curve must comply both with actual experimental data and with theoretical demands. 1. The mathematical expression should show that the velocity of fatality increases very slowly with increase in concentration when very low concentrations of the poison are used. 2. It should show that the velocity of fatality increases very rapidly with an increase in concentration when higher concentrations of poison are used. 3. Finally with still higher concentrations of the poison it should show a less rapid increase in velocity of fatality with increase in concentration. 4. At very high concentrations of poison it should show that the velocity of fatality curve approaches a straight line. See discussion page 43.

When X varies in the equation $Y = \frac{1}{t} = \frac{K_2M+K_1X}{\log_e \left(\frac{M}{M-z} + \frac{K_2Mz}{K_1(M-z)} \cdot \frac{1}{X}\right)}$ and all other factors on the right hand side of the equation remain constant, it is found that when X is very small so that K_1X is very small as compared to K_2M the numerator (K_2M+K_1X) approaches a constant. Thus the value of $\frac{1}{t}$ or Y is controlled by the reciprocal of the logarithm of a number which is controlled by the reciprocal of X, i.e., $\log_e \left(\frac{M}{M-z} + \frac{K_2Mz}{K_1(M-z)} \cdot \frac{1}{X}\right)$. When X is neither very large nor very small as compared to the other factors neither the numerator nor the denominator approaches a constant and Y is controlled by the increase of X in the numerator and the reciprocal of the logarithm of a number which is increased by the reciprocal of X. Finally, when X is very large the denominator $\log_e \left(\frac{M}{M-z} + \frac{K_2Mz}{K_1(M-z)} \cdot \frac{1}{X}\right)$ approaches a constant, $\frac{M}{M-z}$ becomes very large as compared to $\frac{K_2Mz.}{K_1(M-z)} \cdot \frac{1}{X}$. Thus Y is controlled by the increase of X in the numerator (K_2M+K_1X) since K_1X is large as compared to K_2M. From these three conditions it is seen that the value of Y at first increases very slowly, being controlled by the reciprocal of the logarithm of the reciprocal of a number. After this it increases more rapidly, being increased by the same factor as the first and in addition is increased by a multiple of the number itself, i.e., numerator (K_2M+K_1X). Finally, in the third case Y increases only by some multiple of the number since X is very large as compared to $\frac{K_2Mz}{K_1(M-z)}$ the expression $\frac{K_2Mz}{K_1(M-z)} \cdot \frac{1}{X}$ approaches zero. Thus it is seen when $\frac{1}{t}$ or Y is plotted as ordinate and X as abscissa a curve, the velocity of fatality curve, will be formed which at first has a very gradual rise followed by a rapid rise which again is followed by a less rapid rise depending on the value of K_1X which finally approaches a straight line at very high values of X. This curve

will approach a straight line where the direction of the curvature changes (See the portion A to B, curve CABG, Figure 1). The nearness with which this portion approaches a straight line depends on the values of M, K_1, and K_2 as compared to X. Thus the theoretical velocity of fatality curve complies with the actual experimental velocity of fatality curve.

The above equation though conforming in a general way to the experimental data, is doubtless incomplete. It is only an attempt at a mathematical expression which might be taken to represent the experimental data and should be corrected for other factors not taken into consideration here.

The validity of the equation is further emphasized by the work of Burge (1917) in which he shows that both ether and chloroform not only destroy the catalase of the blood of an anesthetised animal but also inhibit the production or the liberation of the catalase by the liver. In other words there are two factors. One is the effect of the poison in inhibiting the liberation or production of an enzyme and the other is the continuous action of the poison in destroying the enzyme which has already been formed or liberated. Both these factors are expressed in the theoretical equation.

A mathematical study of the equation shows that as X decreases in value, Y becomes zero. This value of X corresponds to the threshold of toxicity concentration. When X is decreased below this point, Y becomes imaginary. This is in keeping with experimental data, as very small amounts of certain active substances do not inhibit but stimulate metabolic processes.

COMPARISON OF CURVES OBTAINED BY OTHER WORKERS

Warren (1900) in his work on Daphnia was first to call attention to the fact that when aquatic animals are killed by placing them in solutions of a toxic substance if the survival time is plotted as ordinate and the concentration of the solution as abscissa, the points when joined will form a curve closely resembling an equilateral hyperbola. Warren considered all of his data as complying with this curve. Either he experimented with solutions which would fall within the concentrations where the velocity of fatality curve approaches a straight line or he disregarded data obtained outside this range of concentrations. Warren saw the similarity between his curves and the curves representing Boyle's law and explained his results by supposing that the toxicity of a substance above a definite amount (the writer's theoretical threshold of toxicity concentration) depended upon the number of molecules which beat on the body of the Daphnia per unit of time. Ostwald (1905, 1907) was next to call attention to a similar curve formulated from data obtained by killing Gammarus in different concentrations of sea-water or the constituents of sea-water. Ostwald, disregarding the extremes of his data, claimed that the curve was not an equilateral hyperbola and applied the modified absorption formula, $tC^m = K_1$ or $t(C\text{-}n)^m = K_1$ (Ostwald 1907) (see page 33). Křiženecký (1916) next noted the resemblance to that of an autocatalytic curve of a curve which he obtained by determining the time required for an annelid worm, *Enchytraeus humicultor*, to recover in ordinary tap water after having been in a solution of a toxic substance for one minute, when time was plotted as ordinate and concentration of the solution as abscissa. The curve obtained by Křiženecký resembles the writer's velocity of fatality curve. Křiženecký explained his results in terms of osmotic pressure. Clayberg (1917) called attention to such a curve and called it an hyperbola but made no attempt to show how nearly it approached an hyperbola or its variations. None of these workers have seen the significance of the entire curve nor did they construct what is designated by the writer as the velocity of fatality curve. Neither did they suggest the possibility of its utilization in physiological testing nor attempt to connect their entire data with life processes. Gregersen (1916) in his investigation on the antiseptic value of gastric juice found that the disinfecting value of gastric juice was in direct proportion to the free acidity and that the product of the survival time of the bacteria and the titration number was almost constant. This in fact represents an equilateral hyperbola but such a relation was evidently not noted by Gregersen. Warren also showed that when the survival time of Daphnia killed in a constant concentration of a toxic substance at different temperatures was plotted as ordinate and temperature as abscissa a similar curve was formed.

Krogh (1914, 1914a) and Sanderson and Peairs (1913) almost at the same time and independently of each other noted that a similar curve was formed when rate of development of eggs of frogs, insects and sea-urchins and also larvae and pupae of insects was plotted as ordinate and temperature as abscissa. Sanderson and Peairs determined the reciprocal curve which they designated as the rate of development and considered it a straight line crossing the X-axis at the actual zero of development. They disregarded the variations from a straight line at the extremes altogether and made all calculations on the assumption that the temperature above their theoretical zero times the time required for the organism to pass through certain stages of development is a constant. Krogh in his work called attention to the variations of the rate of development begun below the point at which the straight line cut the X-axis, i.e., the lowest temperature at which development will take place is below the theoretical temperature for the initiation of development. Reibisch (1902) called this the threshold of development.

Finally, Osterhout has shown that the dying curve of Laminaria when killed in an NaCl or a $CaCl_2$ solution of the same conductivity as that of sea-water as shown by the fall of electrical resistance after having passed the point of stimulation of the latter salt follows the course of the same general curve when resistance is plotted as ordinate and time as abscissa.

The close similarity of the curves found by different workers in entirely different fields suggests that the extremes of the reciprocal curves should be more thoroughly investigated. The possibility is that no portion of the curve is a straight line. This supposition is evidenced by the equation $1/t=(MK_2+K_1X)/\log_e\left(\frac{M}{M-z}+\frac{MK_2z}{K_1(M-z)}\cdot\frac{1}{X}\right)$. This is further emphasized by the fact that Ostwald's data fits almost equally well the formulae $y(x-a)=k$, i.e., $1/t=(x-a)/K=K_1(x-a)$ and $t(C-n)=K_1$, i.e., $\log_e t+m\log_e(C-n)=\log_e K_1$. These two equations represent hyperbolae of entirely different orders and are never similar except when $m=1$. Thus the evidence is that neither of the two equations fits exactly. But for all practical purposes in pharmaceutical work and in insect pest prediction (Shelford 1918) the equation $1/t=y=K_1(x-a)$ can be considered as holding in very narrow limits i.e., when the temperature to which the insect pest is subjected is never below the temperature at which the velocity of development curve ceases to approach a straight line; otherwise, some modification of the equation used by entomologists must be employed.

DISCUSSION OF DATA

All substances investigated show very striking similarities in their toxic activities on the goldfish. 1. All substances show a slow relative increase in rate of velocity of fatality with increase of substance used when time required for fatality is above 720 minutes. 2. The greatest rate of increase of velocity of fatality occurs when amounts of substances used will kill the goldfish in periods from about 45 minutes to about 210 minutes with only a few exceptions. (Table XXIV). 3. The rate of increase of velocity of fatality is less rapid with increase in concentration of the substance used when the time required to kill the goldfish is less than about 45 minutes. 4. When the velocity of fatality is plotted as ordinate and normality or the amount of substance used per liter as abscissa, the curve thus formed has a very striking resemblance to a monomolecular autocatalytic curve, i.e., it is a logarithmic curve. HCl and KCN show this character more strikingly than other substances tested as experiments were run over a large range of concentrations. (Figures 14 and 15.) 5. The velocity of fatality of the goldfish is more nearly constant for any given concentration when the concentration of toxic substances used is sufficient to kill the goldfish in a period of not less than 45 minutes nor more than 210 minutes, with very few exceptions. The range for KCN and HCl is from about 90 minutes to about 1800 minutes. This range must be determined independently for each substance. (Table XXIV.) 6. The velocity of fatality curve approaches a straight line for amounts of substances that will kill the goldfish in periods of time just mentioned. This range must be determined independently for each substance by the graphic method from experimental data. (Table XXIV and Figure 1 and explanatory notes.)

From these general results it is seen that when the toxic value or active principle of any substance is to be determined from a pharmacodynamic assay point of view; i.e., to determine the strength of an unknown, only such portions as will kill the goldfish within a range of a certain minimum and maximum time should be used. This range must have been previously determined for each substance to be tested. (See 5 and 6 above and Table XXIV.) An unknown can be determined by any one of five methods. 1. A definite survival time can be utilized, i.e., determine the concentration of the substance that will kill the goldfish in a selected period of time. The survival time of the goldfish for most exact determinations should be within the range of time where the velocity of fatality curve approaches a straight line. This range of time must have been previously determined for each substance to be tested. The portion A to B of the velocity of fatality curve, (Fig. 1 and Table XXIV.) This is a modification of the method proposed by Pittenger and Vanderkleed (1915). 2. A number of goldfish can be killed in the unknown and the average survival time taken and applied directly as ordinate to a survival time curve of the same

substance as that tested which has previously been very carefully prepared by killing a number of goldfish and placed in the hands of workers as a standard curve. For example, if the average survival time of a number of goldfish killed in an unknown LiCl solution was 150 minutes it is seen that the ordinate representing 150 minutes survival time will cut the LiCl survival time curve, LIJM (Fig. 1) at the point R and the normality (0.207 N.) of the LiCl solution. can be read directly from the abscissa. For this method to be most exact the point R must fall within the portion I to J of the survival time curve, i.e., the survival time of the goldfish must be within the portion of the curve where it corresponds to the portion of the velocity of fatality curve that approaches a straight line. 3. A number of goldfish can be killed in an unknown and the average of the survival time determined and data applied to the equation of an equilateral hyperbola, $y(x\text{-}a)=k$, where y=survival time of the goldfish, x= amount of substance used per liter, a=theoretical threshold of toxicity concentration, and k=a constant. For example the average survival time of a number of goldfish killed in an unknown LiCl solution is 150 minutes. By substituting in the equation the value of the constants (a=0.125 N. and k= 12.37 for LiCl) we have $150(x\text{-}0.125)=12.37$. Solving $x=0.207$ N. which is the strength of the unknown. k approaches a constant only when the velocity of fatality curve approaches a straight line. See Figure 1. The deviation of the velocity of fatality curve from a straight line increases progressively as the distance preceeding and following the portion A to B increases, and at the same time the survival time curve deviates from that of an equilateral hyperbola. Curve CABG, Fig. 25, is a graphic representation of this fact [See Shelford (1918) for detail of this curve]. Ordinate of curve represents k [$k=y(x\text{-}a)$]. If the survival time required to kill the goldfish is less than 45 minutes k is greater than 12.37 and if it requires longer than 210 minutes k is less than 12.37. Thus to apply this equation to LiCl the survival time of the goldfish must not be less than 45 minutes nor longer than 210 minutes. This range of survival time must have been previously determined for each substance to be tested. 4. Curve CABG, Fig. 25, when time is interpolated on abscissa (see abscissa at top of graph), can be utilized directly to determine the strength of the unknown, both where k approaches a constant and where k is a variable. Thus to determine the strength of an unknown apply the average survival time of a number of goldfish which have been killed in the unknown to the curve CABG, Fig 25, and read the normality of the unknown directly from the abscissa. For example, if the average survival time of the goldfish killed in an unknown LiCl solution is 150 minutes it will be found that this corresponds to the point R of the curve CABG. Then read directly from the abscissa 0.207 N. which is the strength of the unknown LiCl solution. Again for most exact determinations, concentrations represented from A to B must be used. This curve is of special interest and value in that it emphasizes the variability of k. 5. A few experiments can be run with different concentrations of the substance to be tested and the survival time of the goldfish in each experiment

determined. All concentrations used must fall within the range of concentrations where the velocity of fatality curve approaches a straight line. (Concentrations represented from A to B, curve CABG, Fig. 1). A graph should then be drawn with reciprocal of survival time as ordinate and the ratio of the substance used as abscissa (i.e., the theoretical velocity of fatality curve) on some standard scale. This curve which is practically a straight line and should be drawn as a straight line should then be compared to a standard curve which has been previously prepared from experimental data of the same substance at the same temperature or a curve drawn from data of experiments testing a known solution or standard of the same substance where a standard solution is available. The latter eliminates any error due to seasonal variation, variation in stock of goldfish, physiological state of the goldfish as well as variations in temperature at which the experiments can be run. Then the strengths of the known and the unknown solutions are inversely proportional to the distances from the origin O at which their theoretical velocity of fatality curves cut the X-axis. That is, the strength of the unknown solution is to the strength of the known solution as the distance from the origin O to the point where the theoretical velocity of fatality curve of the known solution cuts the X-axis is to the distance from the origin O to the point where the theoretical velocity of fatality curve of the unknown solution cuts the X-axis. For example, let e=strength of the known solution represented by the theoretical velocity of fatality curve SMP, Fig. 24, v=strength of an unknown solution No. 1, represented by the theoretical velocity of fatality curve SP′M′, and u=the strength of an unknown solution No. 2, represented by the theoretical velocity of fatality curve SP″M.″ Then $v{:}e{:}u = \frac{1}{OP'} : \frac{1}{OP} : \frac{1}{OP''} = \frac{1}{b} : \frac{1}{a} : \frac{1}{c}$ where a=distance

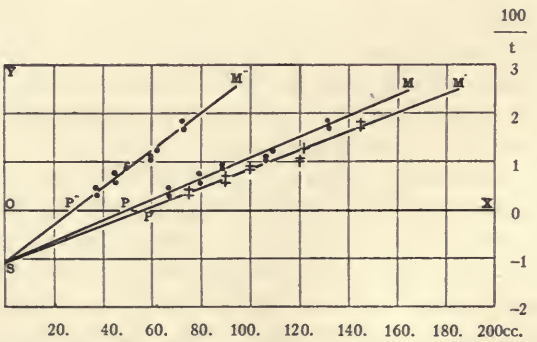

Figure 24. Lithium chloride. Graphs showing method of determining the normality of an unknown LiCl solution.

SPM=Theoretical velocity of fatality curve of a known solution of LiCl
SP′M′= " " " " " " an unknown " " No. 1
SP″M″= " " " " " " " " " " " " 2

TABLE XXXVIII

LITHIUM CHLORIDE

cc. per liter	Survival time of fish in minutes	Reciprocal of survival time of fish 100/t	cc. per liter	Survival time of fish in minutes	Reciprocal of survival time of fish 100/t	cc. per liter	Survival time of fish in minutes	Reciprocal of survival time of fish 100/t
Known Sol.			Unknown Sol. No. 1	Calculated		Unknown Sol. No. 2	Calculated	
127.6	54	1.85	145	54	1.85	72.25	54	1.85
"	58	1.72	"	58	1.72	"	58	1.72
106.9	82*	1.27	121.5	82*	1.27	60.73	82*	1.27
105.6	92	1.08	120	92	1.08	60.0	92	1.08
"	96	1.04	"	96	1.04	"	96	1.04
88	116	.86	100	116	.86	50	116	.86
"	112	.89	"	112	.89	"	112	.89
79.2	141	.71	90	141	.71	45	141	.71
"	179	.56	"	179	.56	"	179	.56
66	234	.43	75	234	.43	37.25	234	.43
"	310	.32	"	310	.32	"	310	.32

*The average survival time of 8 goldfish

This table is based upon calculations from actual experimental data taken from tables I and XXXI

OP, b=OP′, and c=OP″. The theoretical velocity of fatality curves can be better drawn and compared when it is remembered that all theoretical velocity of fatality curves of any one substance when drawn on a definite scale representing the reciprocal of the survival time of the goldfish and variable scales representing amounts of substance used per liter or normality of substance have a definite common point of intersection. That is, if the survival time of the goldfish be plotted as ordinate, and the number of cc. or g. per l. of known and unknown used be plotted as abscissa the curves thus formed would constitute a system of confocal conics of equilateral hyperbolae each being dragged out of position a distance OP, OP′, and OP″ respectively. Therefore the reciprocal curves will all intersect on the Y-axis at the point S. (Fig. 24).

For example two hypothetical unknown LiCl solutions could be determined in the following manner. Make up six solutions from a known LiCl solution as given in the following table and determine the survival time and the reciprocal of the survival time of the goldfish in each solution as recorded. Test the unknown solutions No. 1 and No. 2 as shown in table XXXVIII. Plot a theoretical velocity of fatality curve for each set of data with reciprocal of survival time as ordinate and cc. of a solution used per l. as abscissa 1:100/t is used instead of 1/t to avoid the use of fractions. One block ordinate=1 velocity of fatality. One block abscissa=10 cc. of solution per l.

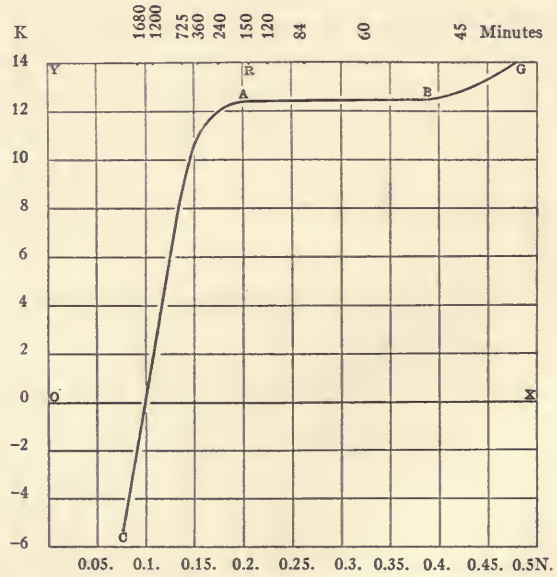

Figure 25. Lithium chloride. Graph showing curve when normality is plotted as abscissa and concentration minus the theoretical threshold of toxicity concentration, times survival time of the goldfish is plotted as ordinate, i.e., k of the equation y(x-a)=k. The graph shows the deviation of k from a true constant. The portion AB is equivalent in range to AB of the velocity of fatality curve Figure 1. y=survival time of goldfish in minutes. x= normality of the LiCl solution. a=theoretical threshold of toxicity concentration=0.125 N. and k=a constant=12.37 which holds only between the two points A and B.

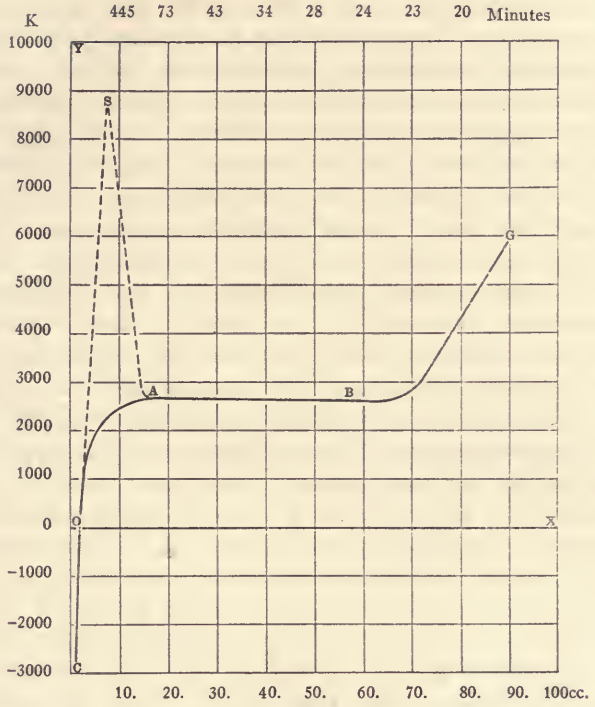

Figure 26. Ethyl alcohol. Graph showing the variation of k. Abscissa represents cc. of alcohol per liter and ordinate represents cc. of alcohol per liter minus the theoretical threshold of toxicity concentration times survival time of the goldfish in minutes. The portion AB is equivalent in range of concentration to AB of velocity of fatality curve Figure 17. $a=2.5$ cc. per liter. $k=2625$. The activity of the goldfish represented by the dotted line has not yet been explained.

In Figure 24 the curve SPM=theoretical velocity of fatality curve of known LiCl solution, SP′M′=unknown solution No. 1, and SP″M″=unknown solution No. 2. 2.22 N.=normality of known LiCl solution. Then let v=normality of unknown solution No. 1 and u=unknown solution No. 2.

$$\text{Then } v : 2.22 : u = \frac{1}{OP'} : \frac{1}{OP} : \frac{1}{OP''} = \frac{1}{2.78} : \frac{1}{2.45} : \frac{1}{1.39} = 1.95 : 2{:}22 : 3{:}91.$$

Thus unknown solution No. 1=1.95 N. and No. 2=3:91.

For all methods suggested the temperature must be kept constant throughout the experiment and at the same temperature as that at which the standard theoretical velocity of fatality curve was prepared. These methods will not hold when the temperature varies.

Results which will allow the above interpretation have been derived independently by different workers (but not thus interpreted by them) in different groups of the animal kingdom as well as the plant kingdom. All will stand the above tests. Ostwald (1905) in his work on toxicity of sea-water and the constituents of sea-water on a fresh water isopod (*Gammarus pulex* De Geer) plotted survival time of the isopod as ordinate and proportion of sea-water or its constituents as abscissa. The curve thus formed when examined casually resembled an hyperbola, however he did not designate the curve as such, but stated, "I have not been able so far to figure out a formula that holds for the whole curve." Ostwald did not construct the reciprocal or velocity of fatality curve. When this curve is constructed from his data, (Table, page 72, showing survival times of male and female Gammarus in different proportions of sea-water, 1905) it resembles very closely the velocity of fatality curves shown in Figs. 1 to 20. Doubtless the resemblance would have been more striking had Ostwald tabulated his data which were omitted and had he run a few experiments with higher concentrations, as he states, "it was only from concentrations of 20 parts of fresh water with 80 parts sea-water and higher, that visible toxic effects began to appear. But these figures thus obtained were still so large and varied so much that I excluded them from my experiments." And later he says that the toxicity of all salts investigated increased very slowly at very low concentrations with increased concentration of solution depending on the nature of the salt and at stronger concentrations there was a sudden rise, while at still higher concentrations there was again a slow rise in toxicity. This is in complete accord with data obtained in this investigation. He then suggests that this difference in the rate of increase of the toxicity of a substance with increase in concentration at very low and at very high concentrations was due to the inability to measure very exactly the low concentrations and to inexactness in determining the death point in a very short survival period. Ostwald (1905, 1907) then disregards these extremes and applies to his mean data the absorption formula (Ostwald, 1906) $a=KC^m$ where $a=$ amount of salt absorbed, C=concentration of the solution, and K and m are constants depending on the nature of the salt investigated. He then assumes that the survival time of the Gammarus is inversely proportional to the amount of salt absorbed, i.e., $1/t=a$, thus $1/t=KC^m$ or $tC=K_1$. Later Ostwald (Ostwald and Dernoscheck, 1910) recognized the inability of the formula to fit his experimental data, and revised his formula by substituting $(C-n)$ for C where $n=$amount of the salt tested normally found in the blood of the experimental animal. But it is difficult to see why such a substitution should be made wih substances not normally found in the blood of the animal. This as has been pointed out would be necessary with all substances tested, since all undergo the same variation in toxic activity with variation in concentration as that pointed out by Ostwald. The only exceptions are $CuCl_2$,

$CdCl_2$, and $FeCl_3$. See page 35. Křiženecký (1916) in his work on *Enchytraeus humicultor*, a fresh water annelid, showed that the survival time of these worms in different concentrations of sea-water, when data is plotted as above, gives a similar curve. Křiženecký explained these results as well as the greater part of the toxic effect of the alkali and alkaline earth metals tested by him as being due to osmotic pressure. He then formulates a curve by tabulating results obtained by determining the time required for the worm to recover in ordinary tap-water after being placed in different concentrations of sea-water for one minute. He then states that both curves have the characteristics of curves of autocatalytic processes in that they fall within the province of the theory of the temporal properties of life processes proposed by Ostwald (1908). Loeb (1903) in summarizing his work on a marine Gammarus, states that, "if sea-water be diluted by the addition of distilled water the duration of life decreases at first only slightly in the decrease of the degree of dilution. But as soon as a dilution of ten times is reached an abrupt decrease in the duration of life takes place with further dilution. Whether the curve of the duration of life at this place is discontinued is not yet proven." Data obtained from the work of Loeb and Wasteneys (1913) on the reduction of oxidation of fertilized eggs of sea-urchins by the addition of .01% KCN solution to sea-water gives a similar curve when per cent of loss of rate of oxidation is plotted as ordinate and relative amount of KCN added to the sea-water as abscissa, Fig. 27. The per cent of reduction of the rate of oxidation of an individual sponge as given by Hyman (1916) in her work showing the effect of KCN on the reduction of the rate of consumption of oxygen by a marine sponge also gives a similar curve when per cent of reduction of oxygen consumption is plotted as ordinate and normality of KCN as abscissa (only data on a single

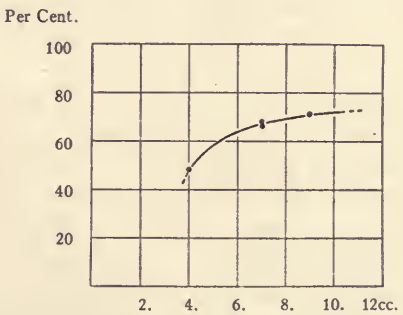

Figure 27. Curve showing the effect of potassium cyanide on the rate of oxygen absorption of fertilized eggs of the sea urchin. Abscissa represents cc. of 0.01% KCN per 50 cc. sea-water. Ordinate represents the per cent of reduction of rate of oxygen absorption below the normal by the potassium cyanide.

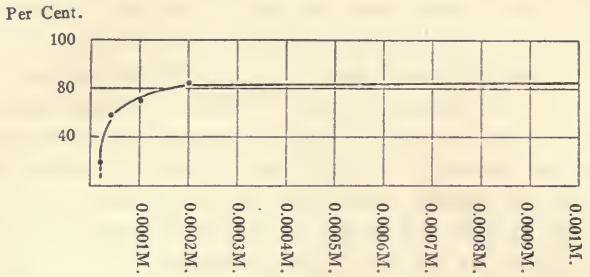

Figure 28. Curve showing the effect of potassium cyanide on the oxygen absorption of a marine sponge. Abscissa represents mols, per liter in sea water. Ordinate represents the per cent of the rate of oxygen absorption reduced by the potassium cyanide below the normal during a unit time (150 minutes). Data taken from Hyman (1916). All data taken from experiments on an individual sponge.

sponge were taken) Fig. 28. The last two calculations are not made on the same basis as the preceding curves but are sufficiently comparable to show that both probably follow the same general law as the toxic activity of salts. A curve formulated from data given in Experiment 1, of Pittenger and Vanderkleed's (1915) preliminary work on the goldfish as a test animal is shown in Fig. 29. This is the same type of curve. This work of Pittenger and Vander-

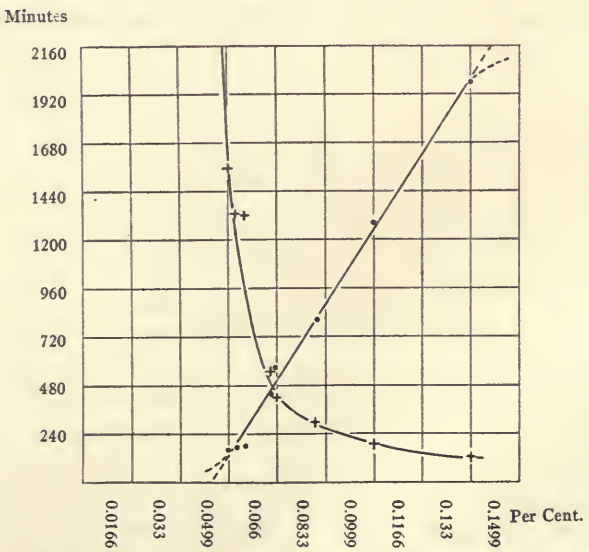

Figure 29. Digitalis. Velocity of fatality and survival time curves. Abscissa represents per cent concentration. Data taken from Pittenger and Vanderkleed (1915).

kleed has been very severely criticized. Many have questioned, as some have put it, "the smooth and elegant results." These smooth and elegant results obtained by these workers are due at least in part to the fact that they were working with solutions which fall within the portion of the velocity of fatality curve that approaches a straight line. (Compare to the portion A to B, curve CABG, Fig. 1.) While the concentrations with which their critics worked possibly fell outside this range of concentrations, it is probable that if their data be reviewed from this point of view it will become more intelligible.

From data obtained in this investigation it is clear that all experimentation for pharmacodynamic assay work should fall within the portion A to B of

TABLE XXXII

THE VARIATION OF THE SURVIVAL TIME OF GOLDFISH

0.318 N. MAGNESIUM CHLORIDE 21° C.		0.284 N. CALCIUM CHLORIDE 21° C.		0.274 N. STRONTIUM CHLORIDE 20.5° C.		0.214 N. BARIUM CHLORIDE 20° C.	
Weight of fish in grams	Survival time of fish in minutes	Weight of fish in grams	Survival time of fish in minutes	Weight of fish in grams	Survival time of fish in minutes	Weight of fish in grams	Survival time of fish in minutes
2.2	73	2.05	110*	2.95	96	1.9	86
2.3	74	2.3	145	2.2	85	2.2	85
2.4	41*	2.4	87*	2.25	73	2.2	89
2.4	75	2.4	95*	2.4	112*	2.3	94*
2.4	76	2.5	96	2.7	89	2.4	91
2.4	77	2.6	93*	2.85	99*	2.4	94*
2.5	78*	2.7	84*	2.9	101*	2.4	101
2.6	77*	3.3	100*	3.0	115*	2.5	100

*Fish was not dead when taken out of solution.

the velocity of fatality curve CABG, Fig. 1. The question then arises by what method can one determine with the least number of preliminary experiments the portion of the curve at which one is working. This can be done either by knowing the extremes of the survival time of the goldfish when the concentrations are within the designated range (A to B) or by running only three preliminary experiments at different concentrations. If the velocity of fatality curve formed by plotting the data from these experiments is convex with respect to the X-axis, the substance should be tested by using solutions falling near the weakest or between the two weaker solutions. If the curve is concave, solutions nearer the strongest or between the two stronger solutions should be used. If the curve is nearly straight and approaches a parallel position to the X-axis the solutions are either too weak or too strong. (These

seem to be the concentrations used by most pharmaceutical workers who have tested the goldfish method of Pittenger and Vanderkleed.) The time required to kill the goldfish will generally tell one whether the solutions are too strong or too weak. See discussion of survival time curve, page 48. If the curve approaches a straight line and is at an angle to the X-axis the solutions are of the most effective concentration. A second method consists in running only one experiment, determining the survival time of the goldfish, and applying this datum to the survival time curve (Curve LIJM, Fig. 1) or to the curve of the constant (Figs. 25 and 26) and reading directly the approximate concentration of the unknown. These curves also show the range of concentrations (concentrations represented by the portions of the curves A to B) most advantageous to use.

TABLE XXXIII

0.246 N. Potassium Chloride

Weight of fish in grams	Survival time of fish in minutes	Temperature Experiment Centigrade	Temperature Stock Centigrade
3.0	16	19.5°	19.5 to 22°
4.3	61	"	" " "
4.7	60	"	" " "
4.75	58	"	" " "
4.9	32	"	" " "
5.1	45	"	" " "
5.05	35	21°	20° " 21.5°
5.4	37	"	" " "
4.4	49	22°	15.5°" 16.5
4.4	79	"	" " "
4.7	42	"	" " "
6.4	69	"	" " "
3.9	46	16.5°	19.5°" 22°
4.9	37**	"	" " "
4.85	37	"	" " "
5.1	83	"	" " "
4.8	57*	"	15.5°" 16.5°
5.3	64	"	" " "
5.75	57	"	" " "
5.8	77	"	" " "

*Fish was not dead when taken out of the solution

**Fish was inactive and on side for about 1/2 minute or less immediately after being placed in solution

TABLE XXXIV

0.2223 Potassium Nitrate

Weight of fish in grams	Survival time of fish in minutes	Temperature Experiment Centigrade	Temperature Stock Centigrade
3.4	31	19.5°	19° to 21°
3.4	35	"	" " "
6.1	70	"	" " "
3.9	45	"	15.5° " 16.5°
4.1	95	"	" " "
5.4	31	"	" " "
4.3	45	15.5°	19° " 21°
4.7	59	"	" " "
4.7	62	"	" " "
4.2	56	"	15.5° " 16.5°
4.8	70	"	" " "
5.1	46	"	" " "

TABLE XXXV

The Variation of Survival Time of Goldfish

Weight of fish in grams	Survival time of fish in minutes	Temperature Experiment	Weight of fish in grams	Survival time of fish in minutes	Temperature Experiment
.250 N. Magnesium Nitrate			.244 N. Magnesium Nitrate		
4.4	87	20°	4.7	40	19°
5.3	47	"	5.2	85	"
5.6	46	"	5.5	85	"
6.0	62	19°	5.5	48	"
6.0	79	"	5.6	45	"
6.1	42	"	5.6	128	"
6.3	59	20°	6.7	42	"
7.3	102	19°	7.1	57	"

SUMMARY OF CONCLUSIONS

1. The survival time of the goldfish (*Carassius carassius* L.) has a very definite relation to the concentration of the solution of the toxic substance used. This relation of the survival time of a goldfish to the concentration of the solution of the toxic substance follows a common general law with a very few exceptions which can be expressed as follows.

a. There is a concentration of each of the toxic substances tested which will just cause the death of a goldfish and concentrations below this will not cause death. This concentration has been designated as the threshold of toxicity concentration.

b. In concentrations of a toxic substance just above its threshold of toxicity concentration the velocity of fatality of the goldfish (as measured by the reciprocal of the survival time of the goldfish) is increased very slowly with increase in concentration of the solution of the toxic substance.

c. In stronger concentrations the velocity of fatality is increased more rapidly with the increase in the concentration of the solution.

d. And finally at very high concentrations the increase of velocity of fatality is again less rapid in proportion to the increase in concentration of the solution.

2. The survival time curve which is plotted by letting ordinate represent survival time of the goldfish and abscissa represent normality or the amount of substance used per l. of water is not an equilateral hyperbola but is logarithmic in function.

3. A curve, the velocity of fatality curve, which is formed by plotting the reciprocal of the survival time of the goldfish as ordinate and concentration of the solution as abscissa resembles a curve which can be expressed by $Y = \frac{1}{t} = \frac{K_2M + K_1X}{\log_e \left(\frac{M}{M-z} + \frac{K_2Mz}{K^1(M-z)} \cdot \frac{1}{X} \right)}$ M = normal rate of metabolism of a goldfish, z = loss of rate of metabolism of the goldfish when in a solution a toxic substance, Y = reciprocal of the survival time of the goldfish, and K_1 and K_2 = two constants depending on the nature of the metabolic process or processes involved and the nature of the toxic substance used.

4. The velocity of fatality curve approaches a straight line when the normality or amounts of toxic substance used per l. of water will not kill the goldfish in less than 45 minutes and does not require longer than 210 minutes. Data within these limits when survival time of the goldfish is plotted as ordinate and the concentration of the toxic substance as abscissa forms a curve which approaches an equilateral hyperbola and can be expressed for all practical purposes by the equation $y(x-a) = k$, where a = distance from the origin where

the portion of the velocity of fatality curve that approaches a straight line when prolonged cuts the X-axis and k=a constant. This straight line which is thus drawn has been designated as the theoretical velocity of toxicity curve. The concentration of the toxic substance represented by the point on the X-axis cut by the theoretical velocity of fatality curve has been designated as the theoretical threshold of toxicity concentration.

5. It has been suggested that relative toxicities of substances be expressed by the formula $T = \sqrt{\frac{\tan. \Theta}{a}}$ when only data which fall within the portion of the velocity of fatality curve which approaches a straight line are used. Θ=angle made by the theoretical velocity of fatality curve cutting the X-axis and a=the theoretical threshold of toxicity concentration of the substance tested. This expression does not represent either the absolute or the exact relative toxicities of the substances since it is based only upon the portion of the velocity of fatality curve which approaches a straight line, but has been chosen since it is a natural criterion and not an arbitrary one.

6. Four modifications of a definite survival time of the goldfish method of pharmacodynamic assay work as suggested by Pittenger and Vanderkleed have been proposed.

a. A definite survival time of the goldfish can be employed provided that the concentration of the substance to be tested is within the range of concentrations in which the velocity of fatality curve approaches a straight line.

b. The average survival time of a number of goldfish in a solution of the substance to be tested can be applied as ordinate to a standard survival time curve and the strength of the solution can be read directly from the abscissa provided this data falls within the limits of the survival time curve which approaches an equilateral hyperbola, i.e., where the velocity of fatality curve approaches a straight line.

c. The average survival time of a number of goldfish killed in a solution of the toxic substance to be tested can be substituted in the equation $y(x\text{-}a) = k$ and the value of x determined which will be the concentration of the solution of the substance tested, provided the survival time of the goldfish is within certain maximum and minimum of survival time, the reciprocals of which when plotted as ordinate and the concentrations of the solutions used as abscissa will approach a straight line, y=survival time of the goldfish, x=concentration of solution of the toxic substance in which the goldfish are killed, a=the theoretical threshold of toxicity concentration, and k=a constant depending upon the substance tested.

d. The average survival time of the goldfish killed in a solution of the substance to be tested can be applied to a graph which has been prepared to show the values of k in different concentrations of the substance with survival time interpolated at the top of graph and the concentration of the solution can be read directly from the abscissa. (See Figs. 25 and 26.)

7. A fifth method of pharmacodynamic testing has been suggested which consists of comparing the velocity of fatality curve of an unknown solution with that of a known solution of the same substance. The strengths of the solutions are inversely proportional to the number of cc. per l. of the original solutions or the number of grams per l. of the two substances required to make up a theoretical threshold of toxicity concentration of the substance.

8. A rise in temperature increases the toxic activity of a substance which probably follows the same general law as that of the activity of toxic substances. This has not been proven and needs further investigation.

BIBLIOGRAPHY

ALLEE, W. C.
1916. Chemical Control of Rheotaxis in Asellus. Jour. Exp. Zool., 21:163-198.
BAKER, W. F.
1913. How Far Variations in Frogs can be Obviated by the Use of Ouabain. Lilly Sci. Bull., ser. 1, no. 3, pp. 71-79.
BERNARD, C.
1857. Leçons sur les effets des substances toxiques et médicamenteuses. Paris.
BERT, P.
1871. Sur les phénomènes et les causes de la mort des animaux d'eau douce que l'on plonge dans l'eau de mer. Compt. rend. acad. sci., 73:382-385; 464-467.
BINET, P.
1892. Physiologie Experimentale. Sur la toxicité comparée des métaux alcalins et alcalino-terraux. Compt. rend. acad. sci., 115:251-253.
BLAKE, J.
1883. On the Connection between Physiological Action and Chemical Constitution. Jour. Physiol., 5:35-44.
1887. Relation of the Chemical Constitution of Inorganic Compounds to their Physiological Action. Chem. News, 55:110-111.
BOTKIN, S.
1885. Zur Frage über den Zusammenhang der physiologischen Wirkung mit den chemischen Eigenschaften der Alkalimetalle der ersten Gruppe nach Mendelejeffe. Centr. albl med. Wissensch., 23:848.
BULLOT, G.
1904. On the Toxicity of Distilled Water for the Fresh Water Gammarus. Suppression of This Toxicity by the Addition of Small Quantities of Sodium Chloride. Univ. Cal. Publ. Physiol., 1:199-217.
BURGE, W. E.
1917. Why Chloroform is a More Powerful and Dangerous Anesthetic than Ether. Science, n.s., 46:618-620.
BURGE, W E. and BURGE, E. L.
1914. The Rôle of Nascent Oxygen in Regulating the Activities of Enzymes in Animals and Plants. Amer. Jour. Physiol., 34:140-148.
1915. The Protection of Parasites in the Digestive Tract against the Actions of the Digestive Enzymes. Jour. Parasit., 1:179-183.
CHILD, C. M.
1915. Senesence and Rejuvenescence. Chicago.
CLARK, J. F.
1899. On the Toxic Effect of Deleterious Agents on the Germination and Development of Certain Filamentous Fungi. Bot. Gaz., 28:289-327.
CLAYBERG, H. D.
1917. The Effect of Ether and Chloroform on Certain Fishes. Biol. Bull., 32:234-248.
DRIESCH, H.
1909. The Science and Philosophy of the Organism. London.
FAGGE, C. H. and STEVENSON, T.
1866. On the Application of Physiological Tests for Certain Organic Poisons and Especially Digitaline. Guy's Hospital Rep. Lond., 12:37.
GARREY, W. E.
1905. The Osmotic Pressure of Sea-water and the Blood of Marine Animals. Biol. Bull., 8:257-270.

1915. The Resistance of Fish to Changes of Osmotic Pressure and Chemical Conditions. Amer. Jour. Physiol., 39:313-329.

GEPPERT, J.

1889. Ueber des Wesen der Blausäurevergiftung. Zeit. klin. Med., 15:208-242, 307-369.

GREGERSEN, J. P.

1916. Untersuchungen über die antiseptische Wirkung des Magensaftes. Centr. Bakt. Par., Orig., 77:353-361.

GUNN, J. W C.

1914. The Influence of Temperature on the Action of Strophantin on the Mammalian Heart., Jour. Pharm. Exp. Ther., 6:39-44.

HASKELL, C. C.

1913. The Influence of Temperature on the Results Secured by Houghton's Method of Assay for the Heart Tonics. Lilly Sci. Bull., ser. 1, no. 5, pp. 199-201.

1914. The Physiological Activity of Ergotin and Powdered Extracts of Ergot. Jour. Amer. Pharm. Assn., 3:786.

HATCHER, R. A. and BRODY, J. G.

1910. Biological Standardization of Drugs. Amer. Jour. Pharm., 82:360-372.

HEFFTER, A.

1908. Giebt es reduzierende Fermente im Tierkörper? Arch. exp. Path., Suppl., 59:253-260.

HOPKINS, F. G.

1914. The Dynamic Side of Biochemistry. Report Brit. Assn. Adv. Sci., Birmingham, 1913; 652-668.

HOUGHTON, E. M.

1898. Pharmacologic Assay of the Heart Tonics. Jour. Amer. Med. Assn., 31:959-961.

HYMAN, L. H.

1916. On the Action of Certain Substances on Oxygen Consumption. I. The Action of Potassium Cyanide. Amer. Jour. Physiol., 40:238-248.

JOSEPH, D. R. and MELTZER, S. J.

1909. The Comparative Toxicity of the Chlorides of Magnesium, Calcium, Potassium and Sodium. Jour. Pharm. Exp. Ther., 1:1-26.

KAHLENBERG, L, and AUSTIN, R. M.

1900. On the Toxic Action of Acid Sodium Salts on *Lupinus albus*. Jour. Phys. Chem., 4:553-569.

KAHLENBERG, L. and MEHL, H. F.

1901. Toxic Action of Electolytes upon Fishes. Jour. Phys. Chem., 5:113-132.

KAHLENBERG, L. and TRUE, R. H.

1896. On the Toxic Action of Dissolved Salts and their Electrolytic Dissociation. Bot. Gaz., 22:81-124.

KASTLE, J. H. and LOEVENHART, A. S.

1901. On the Nature of Certain of the Oxidizing Ferments. Amer. Chem. Jour., 26:539-566.

KISSKALT, K.

1915. Über die Beziehungen der tödlichen Dosis zur Oberfläche. Biochem. Zeit., 71:468-478.

KŘIŽENECKÝ, J.

1916. Ein Beitrag zum Studium der Bedeutung osmotischer Verhältnisse des Medium für Organismen. Arch. ges. Physiol., 163:325-354.

KROGH, A.

1914. On the Influence of the Temperature on the Rate of Embryonic Development. Zeit. allgem. Physiol., 16:163-177.

1914a. On the Rate of Development and CO_2 Production of Chrysalides of *Tenebrio molitor* at Different Temperatures. Zeit. allgem. Physiol., 16:178-190.

LABORDE, J. V.
1884. Sur le mécanisme de la mort ou des accidents qui succèdent a l'injection sous-cutanée du chloroforme, à propos d'une Note de M. Bouchard sur le même sujet. Compt. rend. soc. biol., 36:271-278.

LILLIE, R. S.
1902. On the Oxidative Properties of the Cell-nucleus. Amer. Jour. Physiol., 7:412-321.

LOCKE, F. S.
1895. On a Supposed Action of Distilled Water as Such on Certain Animal Organisms. Jour. Physiol., 18:319-331.

LODGE, O.
1913. Continuity. Science, n. s., 38:379-395, 417-430.

LOEB, J.
1902. Studies on the Physiological Effect of Valency and Possibly the Electrical Charges of the Ions. I. The Toxic and Anti-toxic Effects of Ions as a Function of the Valency and Possibly their Electrical Charge. Amer. Jour. Physiol., 6:411-433.
1903. On the Relative Toxicity of Distilled Water, Sugar Solutions and Solutions of the Various Constituents of Sea-water for Marine Animals. Univ. Cal. Publ., Physiol., 1:55-69.

LOEB, J. and WASTENEYS, H.
1913. Is Narcosis due to Asphyxiation? Jour. Biol. Chem., 14:517-523.

LOEVENHART, A. S. and KASTLE, J. H.
1903. On the Catalytic Decomposition of Hydrogen Peroxide and the Mechanism of Induced Oxidations. Together with a Note on the Nature and Function of Catalase. Pt. I. Amer. Chem. Jour., 29:397-437.

MATHEWS, A. P.
1903. The Nature of Nerve Irritability, and of Chemical and Electrical Stimulation. Pt. II. Science, n.s., 17:729-733.
1904. The Relation between Solution Tension, Atomic Volume and the physiological Action of the Elements. Amer. Jour. Physiol., 10:290-323.
1907. The Cause of the Pharmacological Action of Ammonium Salts. Amer. Jour. Physiol., 18:58-63.
1916. Physiological Chemistry. New York.

MATHEWS, A. P. and WALKER, S.
1909. The Action of Cyanides and Nitriles on the Spontaneous Oxidation of Cystein. Jour. Biol. Chem., 6:29-37.

MEEK, S. E. and HILDERBRAND, S. F.
1910. A Synoptic List of the Fishes Known to Occur within 50 Miles of Chicago. Field Mus. Nat. Hist. Publ., Zoology, 7:223-338.

MIYAKE, K.
1916. The Toxic Action of the Soluble Aluminum Salts upon the Growth of the Rice Plant. Jour. Biol. Chem., 25:23-28.

MOORE, W.
1917. Toxicity of Various Benzene Derivatives to Insects. Jour. Agr. Res., 9:371-381.

MOORE, W. and MOORE, J. J.
1917. Studies in Greenhouse Fumigation with Hydrocyanic Acid: Physiological Effects on the Plant. Jour. Agr. Res., 11:319-338.

OSTERHOUT, W. J. V.
1915. The Measurement of Toxicity. Jour. Biol. Chem., 23:67-70.

OSTWALD, W.
1906. Lehrbuch der allgemeinen Chemie, 2 Bd. Leipzig.

OSTWALD, W.
1905. Studies on the Toxicity of the Sea-water for Fresh Water Animals (*Gammarus pulex* De Geer). Univ. Cal. Publ. Physiol., 2:163-191.

1907. Über die Beziehungen zwischen Adsorption und Giftigkeit von Salzlösungen für Süsswassertiere (Gammarus). Arch. ges. Physiol., 120:19-30.
1908. Über die zeitlichen Eigenschaften der Entwicklungsvorgänge. Vortr. Aufsät. Entwicklungsm., Heft 5.

OSTWALD, W. and DERNOSCHECK, A.
1910. Ueber die Beziehungen zwischen Adsorption und Giftigkeit. Zeit. Chem. Ind. Kolloide, 6:297-307.

PAULI, W.
1903. Untersuchungen über physikalische Zustandsänderungen der Kolloide. Beitr. chem. Physiol. Path., 3:225-246.

PITTENGER, P. S. and VANDERKLEED, C. E.
1915. Preliminary Notes on a new Pharmacodynamic Assay Method. Jour. Amer. Pharm. Assn., 4:427-433.

REED, E. D. and VANDERKLEED, C. E.
1908. The Standardization of Preparations of Digitalis by Physiological and Chemical Means. Amer. Jour. Pharm., 80:110-120.

REIBISCH, J.
1902. Ueber den Einfluss der Temperatur auf die Entwicklung von Fisch-Eiern. Wiss. Meersuntersuch. Kiel., N. F., 6:215-231.

RICHARDS, A. N. and WALLACE, G. B.
1908. The Influence of Potassium Cyanide upon Proteid Metabolism. Jour. Biol. Chem., 4:179-195.

RICHET, C.
1881. De la toxicité comparée des différents métaux. Compt. rend. acad. sci., 93:649-651.

RINGER, S.
1882. An Investigation Regarding the Action of Rubidium and Caesium Salts Compared with the Action of Potash Salts on the Ventricle of the Frog's Heart. Jour. Physiol., 4:370-379.
1883. Concerning the Influence of Saline Media on Fish etc. Jour. Physiol., 5:98-115.
1897. The Action of Distilled Water on Tubifex. Jour. Physiol., 22:xiv-xv (Proc).

ROBERTSON, T. B.
1908. On the Normal Rate of Growth of an Individual, and its Biochemical Significance. Arch. Entwickl. Organ., 25:581-614.

ROBIN, A.
1914. Colloidal Sulphur. Répert. Pharm. 70:161-162. (Cited from Chem. Abst., p. 2920.)

SANDERSON, E. D. and PEAIRS, L. M.
1913. The Relation of Temperature to Insect Life. Tech. Bull. N. H. Coll. Ag. Exp. Sta., 7:1-125.

SHAFER, G. D.
1915. How Contact Insecticides Kill. III. Tech. Bull. Mich. Agr. Coll. Sta., 21:1-67.

SHELFORD, V. E.
1917. An Experimental Study of the Effects of Gas Waste upon Fishes, with Special Reference to Stream Pollution. Bull. Ill. State Lab. Nat. Hist., 11:381-412.
1918. Physiological Problems in the Life Histories of Animals with particular Reference to their Seasonal Appearance. Amer. Nat., 52:129-154.

SOLLMANN, T., MENDENHALL, W. L. and STINGEL, J. L.
1915. The Influence of Temperature and Concentration on the Quantitative Reaction of the Heart to Ouabain. Jour. Pharm., 6:533-560.

TOWNSEND, C. O.
1901. The Effect of Hydrocyanic-acid Gas upon Grains and Other Seeds. Bull. Md. Agr. Exp. Sta., 75:183-198.

TROLAND, L. T.
1917. Biological Enigmas and the Theory of Enzyme Action. Amer. Nat., 51:321-350.
VERWORN, M.
1909. Allgemeine Physiologie. Jena.
WARREN, E.
1900. On the Reaction of *Daphnia magna* (Straus) to Certain Changes in its Environment Quart. Jour. Micr. Sci., 43:199-224.
WELLS, M. M.
1914. Resistance and Reactions of Fishes to Temperature. Trans. Ill. State Acad. Sci., 7:48-59.
1915. Reactions and Resistance of Fishes in their Natural Environment to Acidity, Alkalinity and Neutrality. Biol. Bull., 29:221-257.
WOODWORTH, C. W.
1915. The Toxicity of Insecticides. Science, n.s., 41:367-369.
ZIEGER, R.
1915. Zur Kenntnis der Katalase der niederen Tiere. Biochem. Zeit., 69:39-110.

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